

Computational Modeling of Radial Plant Growth and Cell Division Plane Orientation in the Hypocotyl of *Arabidopsis thaliana*

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*Arabidopsis thaliana***

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*That is the beginning of knowledge - the discovery of something we
do not understand.* – Frank Herbert

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Abstract

Vascular plants contribute by far the most to the world's biomass. By expansion of their tissue, mainly through radial growth of stems and trunks, they represent the backbone of our terrestrial ecosystem. This growth originates from vascular tissues where cambium stem cells drive the organism's expansion by the bi-directional production of xylem and phloem tissue. As the cambium stem cells represent only a single, concentric layer of meristematic cells in the tissue, precise regulation of this process is key to the robust morphogenesis of radial plant growth and, thus, its tissue's long-term survival and supply. This work focuses on the underlying mechanical forces present during radial growth and how they guide shape and growth at the cellular and tissue levels. In particular, we investigate the connection between mechanical stress and cell division patterns in the radial growth of the hypocotyl of the model plant *Arabidopsis thaliana*. As plant cells are contained by rigid cell walls that provide both structural support and mechanical feedback to mechanical stresses via cortical microtubules and cellulose microfibrils, such organisms represent formidable model systems to study the role of mechanics on tissue morphogenesis.

To investigate the physics behind tissue growth and cell division, we develop a cell-based vertex model that simulates radial cross-sections of young hypocotyls. We base our assumptions and parameters on existing experimental evidence to ensure the biological relevance of the results. Starting from abstract, artificial tissues, we find distinct stress patterns to arise during radial growth that also persist in more complex and realistic tissue arrangements derived from segmentations of biological samples. We demonstrate that the simulated tissues grow and behave as expected from experimental data, including the ablation of cells and mechanical perturbations of the tissues in both *in planta* and *in silico*. Ultimately, we show that while the mechanical stress pattern is stable under different cell division rules, only divisions in the direction of maximum stress can reproduce the experimentally observed proliferation patterns. Thus, mechanical stress seems to be the major cue for robust bi-directional cell divisions in the hypocotyl.

With the present analysis, we provide pioneering work in the largely unexplored field of studying the mechanics of internal tissue development, experimentally inaccessible by live-cell

imaging. By applying our vertex model to radial growth and division in the hypocotyl, we show the connection of mechanical stress and its possible role in the guidance of the cell division plane, which suggests common mechanisms throughout different plant organs and tissues. The additional insights gained in this context can be applied in agriculture and forestry to increase the fitness and yield of plants and crops in adverse environments where mechanical stress is a limiting factor of robust growth.

Zusammenfassung

Gefäßpflanzen machen mit Abstand den größten Teil der weltweiten Biomasse aus. Durch die Ausdehnung ihres Gewebes, hauptsächlich durch radiales Wachstum von Stängeln und (Baum-)Stämmen, bilden sie das Rückgrat unseres terrestrischen Ökosystems. Dieses Wachstum hat seinen Ursprung im vaskulären Gewebe, wo Kambiumstammzellen die Expansion des Organismus durch die bidirektionale Produktion von Xylem- und Phloemgewebe vorantreiben. Da die Kambiumstammzellen nur eine einzige konzentrische Schicht meristematischer Zellen im Gewebe darstellen, ist die genaue Regulierung dieses Prozesses der Schlüssel für die robuste Morphogenese des radialen Pflanzenwachstums und damit für die langfristige Überlebensfähigkeit und Versorgung des Gewebes. Diese Arbeit konzentriert sich auf die zugrundeliegenden mechanischen Kräfte, die während des radialen Wachstums auftreten, und darauf, wie sie die Form und das Wachstum von Zellen und Geweben steuern. Insbesondere untersuchen wir den Zusammenhang zwischen mechanischem Stress und Zellteilungsmustern im radialen Wachstum des Hypokotyls der Modellpflanze *Arabidopsis thaliana*. Da Pflanzenzellen von starren Zellwänden umgeben sind, die sowohl strukturelle Unterstützung als auch eine mechanische Rückkopplung auf mechanische Belastungen über kortikale Mikrotubuli und Cellulose-Mikrofibrillen bieten, stellen solche Organismen hervorragende Modellsysteme dar, um die Rolle der Mechanik bei der Gewebemorphogenese zu untersuchen.

Um die physikalischen Zusammenhänge von Gewebewachstum und Zellteilung zu untersuchen, entwickeln wir ein zellbasiertes Vertex-Modell, das radiale Querschnitte junger Hypokotyle simuliert. Wir stützen unsere Annahmen und Parameter auf vorhandene experimentelle Erkenntnisse, um die biologische Relevanz der Ergebnisse zu gewährleisten. Ausgehend von abstrakten, künstlichen Geweben stellen wir fest, dass während des radialen Wachstums deutliche Spannungsmuster entstehen, die auch in komplexeren und realistischeren Gewebearrangierungen, welche aus Segmentierungen biologischer Proben abgeleitet wurden, fortbestehen. Wir demonstrieren, dass die simulierten Gewebe wachsen und sich so verhalten, wie es aufgrund der experimentellen Daten erwartet wird, einschließlich der Ablation von Zellen und mechanischer Störungen der Gewebe sowohl *in planta* als auch *in silico*. Letztlich zeigen wir, dass das mechanische Stressmuster zwar unter verschiedenen Zellteilungsregeln stabil

ist, aber nur Teilungen in Richtung des maximalen Stresses die experimentell beobachteten Proliferationsmuster reproduzieren können. Somit scheint mechanischer Stress das Hauptkriterium für robuste bidirektionale Zellteilungen im Hypokotyl zu sein.

Mit der vorliegenden Analyse leisten wir Pionierarbeit auf dem weitgehend unerforschten Gebiet der Untersuchung der Mechanik der inneren Gewebeentwicklung, die experimentell durch die Bildgebung lebender Zellen nicht zugänglich ist. Durch die Anwendung unseres Vertex-Modells auf das radiale Wachstum und die Teilung im Hypokotyl zeigen wir den Zusammenhang zwischen mechanischem Stress und seiner Rolle bei der Steuerung der Zellteilungsebene, was auf gemeinsame Mechanismen in verschiedenen Pflanzenorganen und -geweben schließen lässt. Die in diesem Zusammenhang gewonnenen zusätzlichen Erkenntnisse können in der Land- und Forstwirtschaft Anwendung finden, um die Beständigkeit und den Ertrag von (Nutz-)Pflanzen in ungünstigen Umgebungen zu steigern, in denen mechanischer Stress ein limitierender Faktor für robustes Wachstum ist.

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1

Motivation

Plants are the ubiquitous foundation of life on Earth, transforming sunlight into energy and producing the oxygen we breathe. Just as in the animal kingdom, plants exhibit an astonishing variety of sizes, shapes, and survival strategies, each specialized to the requirements of its habitat¹⁻⁴. From tiny mosses in tropical rain forests adapted to perpetual moisture, over resilient cacti enduring adverse conditions in deserts, to majestic redwood trees in the northern hemisphere only limited in their height by the laws of physics; the diversity of species and distinct features is remarkable. Of utmost relevance for our everyday life is the growth and development of vascular land plants on which we depend not only to provide us with food but also construction materials and clothing. Thus, there is an intrinsic relevance for us to acquire a deeper understanding of plant developmental biology beyond mere scientific curiosity. Such insights will become especially crucial for responding to the changing global climate, requiring plants and agriculture to adapt to ensure food security and sustainable solutions for improving the fitness and yield of crops.

What unites all plants is the remarkable robustness and plasticity in development and growth amongst the individuals of each species⁵⁻¹¹. Not all types of plants emerge from only a single initial cell, as other strategies like vegetative reproduction exist¹². Regardless of their strategies, individual species follow a distinct procedure from embryogenesis to maturity^{13,14}. The shaping of cells, tissues, and organs throughout this process is known as morphogenesis. In plants, the cellular shape is well defined by rigid cell walls that provide structural support bearing the load of their internal (turgor) pressure¹⁵. Mediated by remodeling their walls, cells may respond to mechanical stimuli and molecular signals. This response then may lead to changes in shape due to turgor pressure, cell-cell interactions, and possibly external environmental cues¹⁵⁻²⁵. On a tissue or organ scale, shape may emerge from multiple pathways^{2,20,26-30}. Considering that cell walls are typically not homogeneous materials^{15,31}, anisotropic growth may occur, for instance elongation growth as observed in roots and

stems^{25,32–38}. Differential growth between tissues, i.e. a variation in growth rates has the potential to bend entire structures, for instance in order to optimize sunlight exposure^{26,39–41}. Another mechanism is directed cell divisions which generate highly regular patterns of cell shapes and proliferation in specific directions, creating files of cells, the lineage of which can even be traced back to one single stem cell^{32,42–44}.

All processes that are part of tissue growth and cellular differentiation are fundamental to developmental biology and need to be carefully controlled by any organism, as failure might range from minor dysfunctions to complete incapability of surviving in extreme cases^{7,45–48}. Examples include embryo-lethal mutations⁴⁹, stunted elongation growth⁵⁰ or abnormalities in flower development, rendering the organism sterile⁵¹.

While for the robust regulation and successful coordination of the developmental processes, biochemical signaling plays a significant role, it does not act in isolation. In addition, mechanical forces substantially contribute to the shaping of an organism^{2,20,26–30}. In plant tissues, these forces and arising stresses and strains within a plant tissue are directly influenced by modifications in the mechanical properties of plant cells and their walls. Such changes, in turn, are regulated by biochemical signaling and closely tied to biochemical pathways^{47,52–59}. Consequently, understanding how cells perceive and respond to mechanical and biochemical cues and how they interact is essential to predicting how tissues will organize and how morphogenesis and proliferation dynamics unfold. The natural approach would be to apply non-invasive methods, which have become invaluable for studying these processes. While in contexts of epidermal tissue, this is a very insightful approach^{60–64}, it is limited to only a few cell layers beneath the surface due to the high density of the tissue and low transparency. Thus, applying non-invasive methods for internal tissues or entire cross-sections is highly limited⁶⁵. To gain a deeper understanding of the dynamics behind it, we need to integrate computational modeling with experimental data into our endeavors to interpolate between different stages of development.

Undoubtedly, biochemical signaling plays a crucial role in tissue development. This study, however, focuses on the analysis of the mechanics of plant development. For this endeavor, plant cells and organs provide rich model systems where each cell can be treated as a pressurized vessel with specific material properties and is subjected to mechanical strain and stress^{28,35,57,66–68}.

It is widely accepted and has been repeatedly shown that plant cells have means of stress-sensing^{25,60,69–71}. Because stress is also tied to the shape of an object^{28,30,72}, this also implies a stress sensing, arising from their shape⁷³. An inevitable feedback loop is created between the response of cells to mechanical stimuli and their shape and stress imposed onto them^{29,68}.

Suppose mechanical stress significantly impacts cell and tissue growth. In that case, the natural question arises whether mechanical stresses influence other aspects of plant development beyond sheer growth, like cell division. Historically, however, geometrical considerations were thought to dominate in determining the cell division plane. Léo Errera observed such a connection in 1888⁷⁴. Errera's rule states that cells divide along the plane that minimizes the surface area of the new cell walls, adhering to principles of geometry and mechanical efficiency. While this geometrical bias has been well-documented^{75–79}, recent studies indeed have proposed an influence of mechanical stress on cell division as well^{19,69,80,81}, potentially overriding this default geometric tendency. Thus, the potential link between mechanical stresses and cell division introduces a layer of complexity to how division planes are chosen and opens up new perspectives on plant growth and division dynamics.

An intriguing case is the radial growth of the hypocotyl of *Arabidopsis thaliana*, which serves as the model system of this work. In contrast to Errera's geometry-based division orientation bias, *Arabidopsis* hypocotyls exhibit division along the axial direction of the organ, which is also the long axis of individual cells^{82–84}. Then, at second order, divisions within that direction are either parallel or perpendicular to the organ's surface^{42,44}. Thus, the hypocotyl does not adhere to Errera's rule and provides a unique system to study the role of mechanical forces on tissue development.

Several modeling approaches have been applied to study plant growth, such as finite element methods^{26,46,85,86} and the Hamiltonian-based vertex models^{57,67,68,87–89}. These models aim to explain the emergence of cell shape and proliferation and to predict mechanical forces, stress patterns and division plane orientations. The overall challenge is to ensure the biological relevance of theoretical models, which also require using a set and range of parameters that accurately reflect the real world⁹⁰. Besides these challenges, plant bio-mechanics has made significant advances in recent years through the further improvement of both theoretical modeling techniques and advances in experimental methods, particularly non-invasive imaging⁶⁵ and analysis tools⁹¹. Nonetheless, many questions remain unanswered, and new insights continue to reveal further aspects to study. Most work so far focused on epidermal layers of tissue under tension^{23,25,60} due to the experimental accessibility, leaving internal tissues often untouched³⁵. In this work, however, we aim to develop an approach towards studying internal tissues based on experimental data of Zakieva et al.⁴⁴, establishing a comprehensive model considering the complete picture to the currently possible extent. Our primary objective is to uncover the effect of mechanical forces on tissue growth and the cell division plane orientation in lateral cross-sections of the hypocotyl of *Arabidopsis thaliana*. To achieve this, we employ two-dimensional computer simulations within the framework of a cell-based vertex

model, which allows us to study the role of mechanical stress in radial plant growth. We focus on uncovering pattern formation and its impact on cell division and tissue organization. A key component of this work is expanding the vertex model to include cell growth and topological changes like cell divisions or variations in the mechanical environment. To ensure biological relevance, we use parameters from experimental measurements and incorporate quantitative and qualitative histological analyses to faithfully reproduce the morphogenesis of the hypocotyl in its early development. In addition, we explore the response of the tissue to external mechanical stimuli by introducing a potential barrier to study its effect on the modulation of the mechanical stress pattern and towards topological remodeling. To systematically approach our objectives, this thesis is structured as follows:

In Chapter 2, we will first cover plant anatomy and physiology aspects and introduce hypocotyl as a model organism for studying radial tissue development. Then, we will consider the significance of individual plant tissue constituents and their impact on the biophysics and mechanical forces behind growth and division. Subsequently, Chapter 3 provides a brief overview of the mechanical modeling of plant tissues, where, in particular, we discuss our cell-based vertex modeling framework to simulate lateral cross-section of the hypocotyl during radial growth and division. With the resulting model, preliminary analyses are carried out in Chapter 4 to challenge its performance and robustness and characterize our system. Ultimately, in Chapter 5, we apply the model to the desired biological context of radial growth, carefully deriving our assumptions and initial conditions from experimental data. Finally, in Chapter 6, we discuss our findings, which conclusions we can draw, and open questions that remain to be answered in future works.

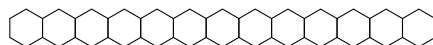
While this work focuses on insights into tissue mechanics and development, its implications extend beyond the specific case of two-dimensional radial growth in the hypocotyl of *Arabidopsis thaliana*. Even though an extension to a three-dimensional analysis of radial growth or biochemical signaling lies beyond the scope of this work, we integrate computational modeling with experimental insights, laying the foundation for understanding how mechanical forces shape tissues and influence cell divisions in internal tissues. These perspectives then might also inspire future research, extending their modeling to the third dimension and the inclusion of biochemical interactions.

2

Plant cells, growth and the biophysics of tissue development

*This chapter covers several key aspects of plant growth and development, laying the biological basis for the present work. To establish this foundation, we introduce basic information on plant cell types and the structure and function of vascular tissues. We then consider aspects of primary and secondary growth in the hypocotyl^a. Our primary focus is on secondary growth, particularly the role of cambium stem cells and cell division in the radial thickening of plants. We discuss the aforementioned topics specifically in the context of the hypocotyl of *Arabidopsis thaliana*, which serves as our plant model system.*

Next, we explore the biophysics behind plant cell growth, starting at the cellular scale with turgor pressure as the driving mechanical force and cell walls as the load-bearing constituents of plant cells. We highlight their involvement in tissue mechanics and further consider the role of mechanical forces and their constraints on plant development. This consideration includes force sensing by cortical microtubules, interactions and feedback mechanisms between them, and arising growth patterns. Lastly, we discuss the overall implications and challenges of considering mechanical forces not only on individual cells but on a tissue scale.



2.1 Plant growth in angiosperms

Growth is a fundamental process in any living organism, yet it significantly differs between the animal and plant kingdoms. While in both cases, the growth of an organism is a result of cell division and individual cell growth, the most notable difference is the presence of rigid

^aFor additional context on primary and secondary growth in the stem and root, see Appendix A.

cell walls as structural elements of plant tissue^{92,93}. Also, animals and humans underlie determinate growth, meaning there's a specific maximum size the organism will reach at which growth stops^{12,94}. On the other hand, plants typically do not have an inherent maximum size. Instead, their growth is only limited by access to water, nutrients, light, or, in the specific case of redwood trees, even gravity. However, the latter limitation feeds back towards the lack of water as too-high trees cannot physically sustain water transport at a certain height⁹⁵. In general, the growth mechanisms and adaptations to environments that a plant shows may highly depend on the individual species. However, certain plant groups with the same characteristics can be distinctly differentiated^{12,93,94}. At a broad level, there are non-plants, such as algae, which lack key characteristics of plant cells, and there are land plants. The latter can be further subdivided into non-vascular and vascular plants (cf. Section 2.1.2). Vascular plants include ferns, gymnosperms, and angiosperms⁹³. This work focuses on the growth and development of angiosperms, i.e., vessel seed plants, where a protective shell surrounds their seeds. This group of vessel seed plants includes basal angiosperms, monocots, and eudicots^b. While monocots are plants with only one embryonic leaf (cotyledon), eudicots and basal angiosperms have two. The shared evolutionary innovation of angiosperms is that they are all flowering plants^{12,93}. The growth of angiosperms generally includes three main developmental steps: embryogenesis, vegetative growth, and reproductive development^{12,93}. This work focuses on late embryogenesis and especially the early vegetative growth phase. Looking more specifically into the growth of monocots and eudicots, on a small scale, all of these stages comprise very complex interactions between mechanical forces and biochemical signaling, including stochasticity, whereas, on the larger scale, in first approximation, we can distinguish between two different types of growth, primary and secondary growth^{9,12,96–98}. These types of growth are both driven by meristems, i.e., tissue of undifferentiated or incompletely differentiated cells that may divide and develop into specific types of tissues or organs of the plant^{12,93,94,99}. In short, primary growth refers to the emergence of new organs, such as shoots, roots, and leaves. It is thus also closely associated with the elongation growth of the plant and its organs, which ensures the establishment of the plant after germination by quickly growing roots to absorb nutrients and water and elevating leaves to provide access to sunlight. Secondary growth, on the other hand, does not produce new organs but is the main source of radial expansion of a plant and is, unlike primary growth, not necessarily present in all angiosperms¹². In the following subsections, we will discuss growth and the different cell

^bHistorically, it was only distinguished between monocots and dicots, where dicots included both eudicots and basal angiosperms. As the evolution of eudicots and basal angiosperms deviated from each other, in more modern botany, they are grouped individually⁹³.

types involved in more detail. It is important to mention, however, that while the information is kept as general as possible, depending on the plant species, the tissue organization and development may differ substantially.

2.1.1 Plant cells and their different cell types

Before we delve into the details of plant growth modes, the specifics of growth in the hypocotyl as a model system, and the biophysics of plant morphogenesis, we first consider some basics of plant cells and cell types. Plant cells, just like animal cells, are highly complex structures of many individual components. The primary component that sets plant cells apart from animal cells is the synthesis of an external cell wall that contains all the other components of the cell^{92,93}. The basic structure of a generic plant cell is shown in Figure 2.1.

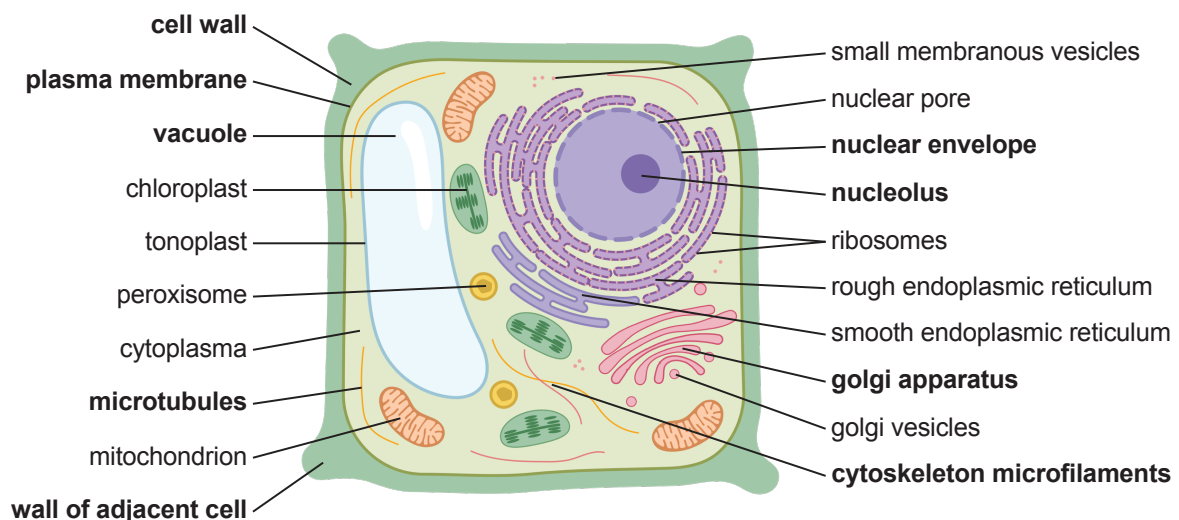


Figure 2.1: Schematic of plant cell components The plant cell as a living cell contains many organelles that control its metabolism and determine its function and properties. From a mechanical perspective, the most important organelles for cell growth are the cell wall, the plasma membrane, the vacuole, and the cytoskeleton¹². Organelles that we discuss or at least touch upon in this work are highlighted in bold print. Created in BioRender. Höfler, M. (2024) <https://BioRender.com/r14b784>

While we will not discuss all the different components in detail, some play a vital role in cellular biophysics during cellular growth and division, which we will explain throughout the following sections. One key component in plant cell biomechanics is the cell wall (Section 2.2.2), which provides structural integrity¹⁵. It resists the high internal turgor pressure (Section 2.2.1) that arises from water intake into the vacuole, driving the cellular

expansion¹⁶. Another integral constituent of plant cells is cortical microtubules, which we discuss in detail in Section 2.2.3. They affect the mechanics of cell walls and cell divisions as they guide the deposition of cellulose^{15,100,101}. For this reason, microtubules are a key factor in cell divisions and in the synthesis of new cell walls^{12,102,103}. All of these aspects, we will explore further in the following sections of this chapter.

Considering, for now, plant cells as building blocks of tissue, the structure of individual cells and the size of specific organelles may substantially differ, depending on the cell's purpose¹². Beyond such differences, plant tissues and organs are generally composed of three main types of cells: parenchyma, collenchyma, and sclerenchyma (Figure 2.2)¹². These cell types differ in structure, function, and mechanical properties. Understanding their properties and differences is beneficial for further considerations regarding the cell types and tissues we aim to study in this project. Therefore, we will now briefly introduce the different cell types before discussing the primary and secondary growth processes.

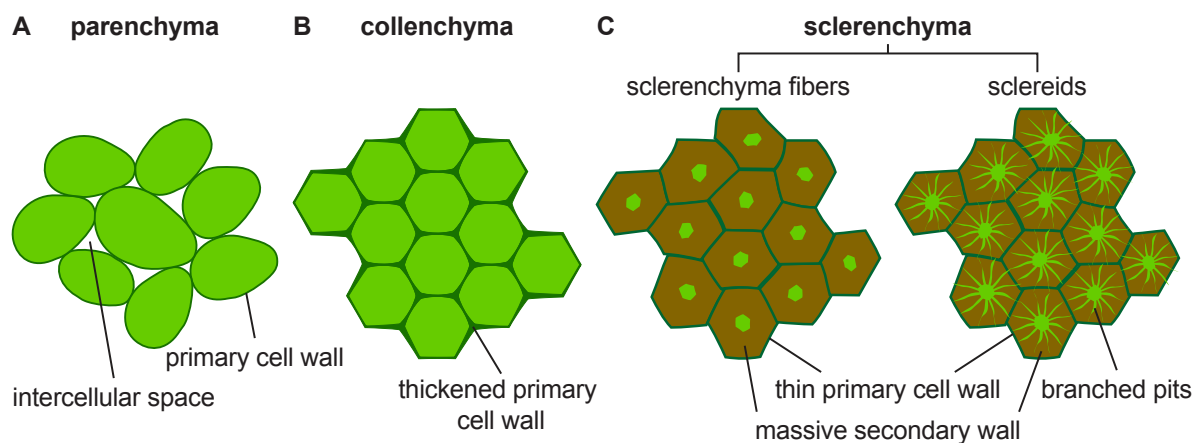


Figure 2.2: The three basic types of plant cells: (A) Parenchyma cells have a characteristic intercellular space in between them and thin, primary cell walls. (B) Collenchyma cells can be recognized by the lack of intercellular space but locally thickened primary cell walls at the junctions between cells. (C) Sclerenchyma, unlike the other two, are dead cells at maturity, and the majority of their volume is filled by a very thick, lignified, secondary cell wall (cf. Section 2.2.2). Sclereids, also called stone-cells, are a sub-type of sclerenchyma and also have additional branched pits, allowing lateral intercellular transport.

Parenchyma

Parenchyma cells (Figure 2.2A) are by far the most common type of plant cell, making up about 90% of cells, and are found in many tissues in a plant¹². Of all the types, they are the most classical representatives of plant cells. They essentially have the structure shown in Figure 2.1 up to specific differences, depending on their specialization and purpose. Parenchyma cells are found in various plant tissues. They are a major component of ground tissues, such as the pith

and cortex in stems and roots (cf. Appendix A). Additionally, parenchyma cells are present in primary and secondary meristems, where they contribute to growth. Within vascular tissues, they are further found in the phloem and as sclerified parenchyma within the xylem^{12,99,104}. The latter two, we discuss in Section 2.1.2.

Parenchyma are living cells with a large variety of functions and are unique in that they are the only one of the three types of plant cells that can undergo mitotic division and retain a high level of developmental plasticity¹². Often, parenchyma cells are simple and not fully specialized, like ground tissue, representing mostly filler tissue. Despite that, they are capable of high specialization, contributing to important metabolic tasks such as photosynthesis, nutrient storage, assimilation, and respiration or secretion^{12,99}. Parenchyma cells have only thin, primary cell walls with intercellular spaces that facilitate the diffusion of water, gases, and minerals involved in the plant's metabolism. Some simple tissues like aerenchyma or chlorenchyma may only consist of parenchyma cells, whereas other tissues like the vascular tissue (Section 2.1.2) are a combination of different cell types. The abundance of parenchyma cells provides an advantage towards survival as they exhibit totipotency¹², i.e., the ability to divide and differentiate into any other cell type. As a result, even a single parenchyma cell can, in principle, divide and differentiate towards a complete plant again^{12,105}.

Collenchyma

While in non-vascular, herbaceous plants (bryophytes), parenchyma cells may be sufficient, vascular plants like angiosperms require additional structural support and nutrient transport capabilities, which is the task of collenchyma and sclerenchyma^{12,106}. Collenchyma (Figure 2.2B) are relatively rare, only making up about 1% of plant cells¹². Like parenchyma, collenchyma are living cells that only have primary cell walls. Typically, these cells can be found in tissues that are rapidly growing, such as elongating stems, where they provide mechanical stability while still being stretchable enough not to hinder elongation^{12,106}. The structural support of collenchyma results from local thickening of the primary cell walls during elongation in a layered fashion, especially at junctions between cells. These thickened primary walls consist mainly of the polysaccharides cellulose and hemicellulose, with a substantial fraction of water embedded in them¹⁰⁶. Due to their layered arrangement, even birefringence may be observed in such tissue¹². Despite their more specialized function, collenchyma cells also retain a large degree of developmental flexibility as the local thickening of their primary cell walls is reversible¹².

Sclerenchyma

Perennial, wood-like plants typically require further support from rigid secondary cell walls provided by sclerenchyma cells (Figure 2.2C). Unlike parenchyma and collenchyma, these cells are typically dead at maturity, and their structural stability is provided by their sturdy cell walls rather than their turgor pressure^{12,107}. Sub-classes of sclerenchyma are fibers, sclereids, and water-conducting sclerenchyma, especially the latter bearing a vital role in the vascular tissue, more closely described in Section 2.1.2.

Fibers (Figure 2.2C, left) are typically clustered, elongated cells. They have limited nutrient transport capabilities but provide protection and structural support via deposition of the biopolymer lignin in their cell walls, leading to their woody rigidity¹². Depending on the context, cells reinforced by lignin deposition are called lignified or sclerified cells. Fibers can occur both inside (xylary) or outside vascular bundles (extraxylary). Often, they are associated with primary phloem and secondary xylem (cf. Appendix A.1, A.2). Despite the rigidity of fiber cells, their cell walls may retain some elasticity, providing robustness against stretching and bending. Well-known everyday examples of fiber tissue are hemp, jute, and flax¹².

Sclereids (Figure 2.2C, right), also called stone-cells⁹⁹, are comparatively small and have a higher shape variety than fibers and water-conducting sclerenchyma. They also form clusters of cells with heavily lignified cell walls and are thought to be coupled regarding their development to the expression of the plant hormone auxin¹². There exist several subforms of sclereids, but their shared feature is the overall toughness, providing hard shells or coats of seeds, for instance, or sealing wounds by sclerification where parenchyma cells develop a thick secondary cell wall, transforming them into sclereids. For intercellular transport, sclereids often form branched pits^{12,99}.

Water-conducting sclerenchyma or tracheary elements are central for the function of xylem tissue. In angiosperms, we can distinguish between tracheids that provide long and narrow channels or vessel elements that are shorter and wider. They are the main conduit for water transport from the roots to the aerial organs¹².

While these fundamental cell types are distinct from each other and carry out different tasks, they do not act in isolation. Instead, different cell types may contribute to a tissue. In the following section, we will explore the roles of parenchyma, collenchyma, and sclerenchyma in plant structure and function in the context of vascular tissue, the focus of this project.

2.1.2 Vascular tissue in the hypocotyl

Like in trees, a typical feature of eudicots is their layered tissue structure, consisting of concentric rings, where each ring represents a specific type of tissue (cf. Figures 2.4 and 2.5). Some of these layers are referred to as vascular tissues. Vascular tissues represent a significant evolutionary development, allowing higher plants to grow taller and survive in many different environmental conditions by their increased mechanical support and capabilities of water and nutrient transport^{82,108}. Such transport is ensured by two central conduction systems: the xylem and the phloem. Primary xylem and phloem are generated by the shoot apical meristem and the root apical meristem; secondary xylem and phloem by the vascular cambium¹². Here, we only consider secondary vascular tissues though (cf. Section 2.1.3), for their relevance towards the present work^c. Xylem is a complex tissue of different cell types to provide structural support for the developing plant and water conduction¹². Phloem tissue represents the other type of conduction system, which comprises the transport of sugars, transporting the products of photosynthesis^{12,107}. The third tissue is the vascular cambium, which is only present in eudicots and bi-directionally produces xylem and phloem tissue during secondary growth^{42,58}. In the following, we discuss these different tissues, their composition, structure, and purpose, especially concerning the secondary growth of the hypocotyl of *Arabidopsis thaliana*; hence, we will not consider primary tissues.

Xylem

The xylem is one of the two conduction systems of plants and serves several purposes, mainly water conduction, dissolved minerals transport, and structural support^{12,84,107}. It allows a plant to transport water from the soil to the atmosphere, which relies on water evaporation, i.e., transpiration from the leaves and the cohesive and adhesive properties of water¹⁰⁴. The xylem in angiosperms consists of two cell types: parenchyma and the sclerified imperforate and perforate tracheary elements.

Tracheary elements have thick secondary cell walls and can be distinguished between three types. Tracheids contribute towards water and mineral transport, while fiber-tracheids and libriform fibers contribute mainly to the structural stability of the plant and are little involved in water conduction^{12,104,107}. All these elements are long and narrow with tapered ends and typically have pits of different numbers and shapes at their side walls. Tracheids carry a lot of

^cNotably, secondary phloem is typically only annual tissue due to xylem expansion. Because xylem tissue is located at the center of the plant, its growth exerts pressure on the outer tissue layers, including the phloem and epidermis. Ultimately, due to the mechanical sturdiness of the expanding xylem, the outer layers yield and are either ruptured or crushed and ultimately replaced in the subsequent year.

circular pits that allow for lateral water transport between cells. They typically reside next to perforate tracheary elements, also known as vessel elements¹².

Vessel elements have so-called perforation plates located at the end walls of cells and can be of different shapes, like simple holes or ladder-like structures that facilitate water transport. These perforation plates allow the plant to stack multiple cells to form a continuous tube as the end walls degrade upon apoptosis, i.e., programmed cell death. After apoptosis, only the lignified cell walls persist. Still, the formation of the secondary cell wall continues, further increasing the mechanical stability of the cell as a contribution to the overall stability of the plant while still serving the purpose of a conductive system¹⁰⁷. Thus, vessel elements allow for efficient end-to-end water transport, optimizing the axial water conduction by forming a larger vessel from individual elements^{12,107}.

Xylem parenchyma, unlike the other xylem cell types, are living cells derived from the vascular cambium that can be either parallel to the elongation axis or represent ray parenchyma oriented towards the radial axis. Their purpose is, for example, among storage, pathogen defense, wound healing, and water balance^{12,84,107}. For instance, if transpiration in tracheary elements is blocked because the air or the soil is too dry to provide enough water and water vapor inside the plant tissue is blocking the water conduction, this may be restored by water supplies from parenchyma to fill the tracheary element again¹².

Phloem

Phloem tissue represents the second conduction system of plants, which transports mainly nutrients that are products of photosynthesis, i.e., sugars. It transfers these products from their production source to sinks where they are required. One speaks of autotrophic tissue, i.e., tissue that generates organic compounds from abiotic energy sources like leaves, and of heterotrophic tissue that consumes those compounds, such as roots and developing fruit¹⁰⁴. Like the xylem, the phloem is a tissue consisting of multiple cell types of specialized structure and function tied to their purpose, namely sieve tube elements, companion cells, parenchyma, and fibers¹². Apart from the phloem fibers typically found outside of other phloem cell types, the remaining three are living cells of parenchyma type (cf. 2.1.1) that are the primary conducting cells. Sieve tube elements and companion cells only transport nutrients axially, though, while radial transport, especially during the growth of the periderm, is carried out by phloem ray parenchyma^{12,104}.

Sieve tube elements transport and distribute sugars, amino acids, and hormones in a plant and are narrow and elongated just as xylem vessel elements or tracheids^{12,104}. In analogy to the xylem vessel elements' perforation plates, sieve tube elements also possess sieve plates in

their end walls and sieve areas with sieve pores in their lateral walls, the latter being enlarged plasmodesmata, i.e., cell-to-cell connections to link different sieve tube elements to both their ends and sides^{12,104}. Once sieve-tube elements reach maturity, partial apoptosis occurs, after which they rely on companion cells to survive, forming a sieve-tube-element-companion-cell-complex. This arrangement is required because the sugar transport depends on active transport, unlike the physically driven water transport in xylem vessels¹⁰⁹.

Companion cells are located next to each sieve tube element and, among other roles, contribute mainly towards metabolic support and loading or unloading of phloem sieve tubes with photosynthates. Loading and unloading are carried out either by symplastic loading or apoplastic loading^{12,104,109}. The first case represents a diffusion-based loading through the plasmodesmata. Once small sugar monomers reach the sieve tube elements, they polymerize to larger sizes. They are trapped in the vessel elements and transported to a sink cell, where they depolymerize for storage. Apoplastic loading, in contrast, is a fully active process where transport proteins cause a unidirectional transport of sugars from the apoplast into the companion cells and then into the sieve elements^{12,104,109,110}.

Phloem parenchyma contribute towards axial and radial transport (axial and ray parenchyma), coordination of xylem and phloem, and nutrient storage^{12,104}. Ray parenchyma connect the tissue over its entire radius by forming xylem rays at the inner layers and phloem rays at the outer layers. Both descend from ray initials created by the vascular cambium, and their interplay allows for a radial supply of water from the xylem ray parenchyma towards the phloem by symplastic transport. Besides the water transport of rays, phloem parenchyma also stores starch and other compounds that are distributed radially and axially. In combination, phloem parenchyma and xylem parenchyma provide a transport network for the plant as a whole^{12,104}.

Phloem fibers provide further structural enhancement and protection in addition to xylem tissue and are found throughout the phloem and the periderm. Unlike other cell types, they do not always emerge from the vascular cambium. Phloem fibers are sclerenchyma, i.e., dead cells that stabilize the surroundings of the comparably delicate sieve-tube-elements^{12,104}.

In summary, phloem and xylem collaborate for nutrient transport, according to the Münch pressure flow hypothesis, in such a way that sugars get produced in autotrophic cells like leaves, which is then transported through companion cells or phloem parenchyma towards sieve tube elements. There, the nutrients are loaded into the water and transported downward, where the process is reversed. Nutrients are unloaded and stored via companion cells or phloem parenchyma in heterotrophic cells like roots. The remaining water is then carried

upwards again by xylem vessels to provide a continuous water and nutrient flow^{12,104}.

Cambium

Found between the xylem and phloem tissue, almost exclusively in eudicots, is the vascular cambium stem cell tissue^{12,111}. It is the source of all secondary vascular tissue and bi-directionally produces xylem and phloem cells^{42,58} (see Figure 2.5B), hence is the source of secondary, i.e., radial growth of perennial eudicots. Note that only during very early development do these stem cells divide transversely (along the short axis) to establish a sufficient number of stem cells. After the establishment of all initial structures and with the onset of radial growth, they only divide longitudinally, i.e., in the direction parallel to the stem's axis. This division orientation coincides with the long axis of the cells. Such long-axis divisions are relatively unique to be observed in plant cells^{82–84}. Unlike the xylem and phloem, the vascular cambium consists of only one single layer of meristematic cells located between these tissues. If this cell layer is somehow damaged or removed, it cannot be replaced^{12,84}. It is, therefore, arguably also the most critical part of the plant as the production of additional cells in the radial direction that then contribute to the conduction of water and nutrients solely depends on this single layer. The cambium consists of so-called fusiform initials and ray initials that contribute to the plant's axial and radial transport systems^{12,34,82,112,113}. Fusiform initials produce xylem and phloem to support water and nutrient transport in the longitudinal direction and, thus, are highly elongated cells. In contrast, ray initials give rise to parenchyma cells that contribute to radial sustenance and, therefore, are more uniformly shaped. Fusiform initials furthermore may occur in storied or non-storied arrangements, meaning an alignment of files of cambial initials longitudinally such that they either all start and end approximately at the same height or are arranged somewhat randomly^{12,34,82,112,113}. In *Arabidopsis*, it was shown that both types might exist. However, storied cambium seems to be only present in inflorescence stems¹¹⁴, and in general, non-storied (also non-stratified) cambium is more common among eudicots¹². As shown in Figure 2.5B, cambial initials produce xylem and phloem derivatives that are still capable of dividing yet another time before fully differentiating¹¹⁵, also reducing the risk of mutations arising from cambial stem cells¹². Due to this extended division and differentiation zone, even though the actual cambium is only one cell layer, one speaks of a cambial zone that comprises this mitotic region in the cross-sectional area of the plant. Beyond the vascular cambium, the cork cambium or phellogen counts towards vascular tissues in mature tissues and produces phelloderm inwards and cork or phellem outwards (cf. Figure 2.5A)^{12,34,108}.

2.1.3 Primary and secondary growth in the hypocotyl of *Arabidopsis thaliana*

In general, plant growth takes place in two different growth modes: On the one hand, there is primary plant growth, where the organism increases in length; on the other hand, there is the radial expansion of plant tissue, the secondary plant growth. Both types are driven by cellular growth and meristematic stem cell division. The meristems that drive primary growth are the shoot apical meristem and root apical meristem, emerging from the plant's shoot and roots. Secondary growth is driven, however, by the vascular cambium (Section 2.1.2) and the cork cambium^d. Typically, both of these growth modes take place simultaneously in plant tissue. However, there is one organ where both are temporally displaced, which is the hypocotyl^{116,117}. The hypocotyl, in essence, connects the plant's root and shoot system. During germination, the hypocotyl plays a key role in early plant development and is part of any eudicot. As a shared feature of monocots and eudicots, though, once the plant's embryo has developed and the seed germinates, the first part that extends to the outside is always the radicle from which the primary root develops (Figure 2.3A)¹¹⁸. At this stage, monocots and eudicots start to develop in different ways. In eudicots, the radicle elongates, and from it emerges the root cap with the root apical meristem beneath¹². From this, the primary root or taproot evolves, which might develop additional lateral roots (Figure 2.3B).

This root system is connected to the upper parts of the plants via the hypocotyl, which serves as the interface between the root system and the shoot system, defining the apical-basal embryonic axis (Figure 2.3C)^{14,118}. The name itself is a composed word of the Greek *hypo* and *cotyledons*, meaning "below the cotyledons", which is where the hypocotyl can be located¹². At the very top forms the shoot apical meristem, which by the hypocotyl is driven upwards rapidly after germination to allow for reception of light for self-sustained growth¹¹⁹. Further on, between the hypocotyl and the shoot apical meristem, above the cotyledons, the epicotyl emerges from which the first true leaves, the epicotyledons emerge (Figure 2.3C)¹².

In contrast, the root development of monocots significantly differs from that of eudicots. After an initial set of primary roots during embryogenesis, monocots establish a secondary root system consisting of many so-called adventitious roots that emerge from the stem, which make the root system more fibrous in contrast to the typical taproot round in eudicots^{12,120}. Consequently, the hypocotyl is either absent or very short in many monocots. Instead, they develop a structure called mesocotyl, which elongates and serves the same purpose as the

^dFor additional information on the primary and secondary growth of stems and roots in monocots and eudicots, see appendix A.

hypocotyl during germination, pushing the upper parts of the plant above the soil's surface¹¹⁹.

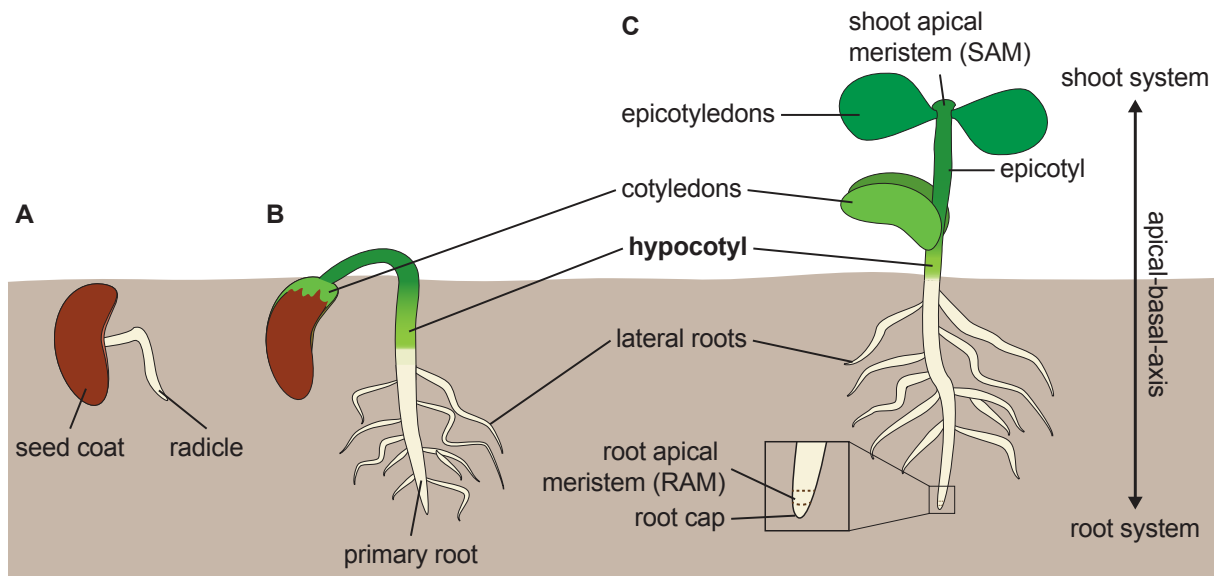


Figure 2.3: Schematic of the embryogenesis of *Arabidopsis thaliana*. (A) From the germinating seed, the radicle as the first embryonic root emerges. (B) The radicle extends into the primary root with additional lateral roots, and the hypocotyl extends upwards to pierce the soil's surface for access to sunlight. Subsequently, the germinated plant sheds the seed coat, and the cotyledons emerge. (C) In the fully developed plant, the apical-basal axis is established with the shoot apical meristem at the top and the root apical meristem at the bottom, connected by the hypocotyl. Beneath the hypocotyl and cotyledons, the epicotyl and the first true leaves, the epicotyledons emerge.

Due to their high sensitivity to sunlight, unlike hypocotyls, the mesocotyls are typically not visible above ground as growth is strongly inhibited in light-grown samples¹²¹. However, a similar level of plasticity is observed in hypocotyl. There, the elongation growth may also immensely vary if grown in an etiolated environment, i.e., without light, potentially leading to elongation tenfold of the one under white light conditions in *Arabidopsis*¹²². Despite its potential to elongate, unlike the root and the shoot, there is no developmental gradient in the hypocotyl¹²³, meaning that the tissue structure is the same along the organ's length. In contrast, in the stem or the root, segments either undergo primary growth or have transitioned already to secondary growth, depending on the position along the apical-basal axis.

While the hypocotyl connects the root and the shoot system, its morphology and developmental patterns are more closely related to the ones of the roots than to the stems, highlighting the hypocotyl's role as a transitional structure bridging the gap between the two^{32,124}. The overall morphology of the lateral cross-section of the hypocotyl of *Arabidopsis* during primary growth and towards secondary growth is displayed in Figures 2.4 and 2.5A.

Instead of vascular bundles, consisting of xylem, cambium, and phloem (see Section 2.1.2),

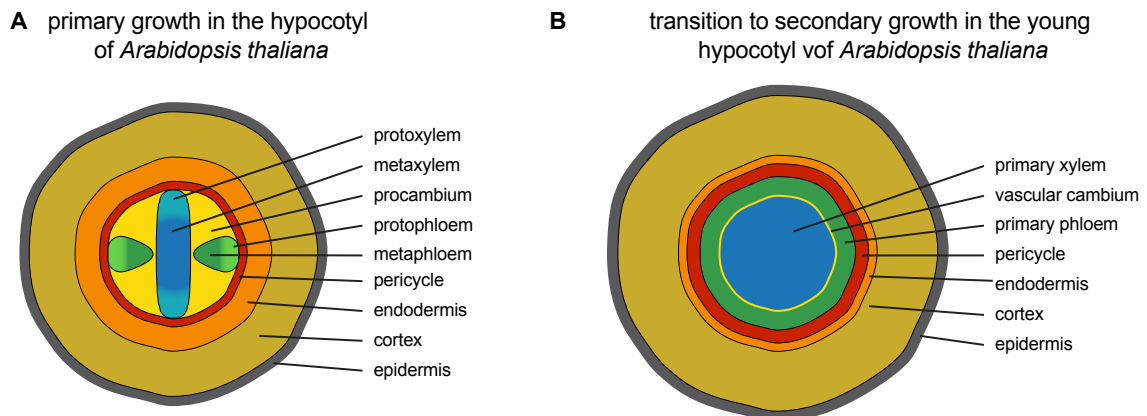


Figure 2.4: Primary growth of the hypocotyl of *Arabidopsis thaliana*: (A) During primary growth, the hypocotyl of *Arabidopsis* features a diarch primary xylem and phloem arrangement, similarly to eudicot roots (cf. Supplemental Figure A.2B) with procambium in between. The pericycle, endodermis, and epidermis surround the vascular tissue. (B) During the transition from primary to secondary growth, the vascular cambium emerges to form a ring of meristematic tissue, surrounded by primary xylem inwards and primary phloem outwards. Subsequently, the vascular cambium produces secondary xylem and phloem.

that are present in the stem (cf. Appendix A), the lateral cross-section during primary growth is composed of four equidistant poles (see Figure 2.4 A), leading to a diarch structure of two poles of each protophloem and protoxylem^{32,107,124–128}. From the latter, early on metaxylem forms by stretching and breaking parts of the protoxylem and replacing it, connecting both protoxylem poles. Procambial cells are found between this primary xylem and phloem. Like in the roots of eudicots, pith is largely absent^{12,123}, and the primary vascular tissue is embedded within other tissue layers towards the organism's surface. These are from inwards to outwards, the pericycle, endodermis, cortex, and epidermis. In contrast to the root, two layers of cortex cells are typically present instead of only one^{125–127}. Still, during primary growth, the procambium proliferates and sets the basis for the vascular cambium, which, as in stems and roots, eventually forms a ring of cambial tissue, leading to the typical cylindrical vascular structure (Figure 2.4B)^{32,107}. As with woody plants, towards the onset of secondary growth in *Arabidopsis*, the pericycle cells begin to divide anticlinally, i.e., perpendicularly to the hypocotyl surface to increase in numbers at the expense of endodermal cells, at last, the ones next to the xylem poles. As a result, over time, the endodermis is entirely replaced with pericycle cells, from which eventually the phellogen emerges. The phellogen then bi-directionally produces the phellem to the outside and the phelloderm to the inside (Figure 2.5A)^{12,126,129}. Due to the additional radial expansion initiated by the phellogen, the cortex and epidermis eventually slough off¹²⁶. As a result, what remains is again the vascular tissue of the xylem, vascular cambium, and phloem, surrounded by periderm as in the root and

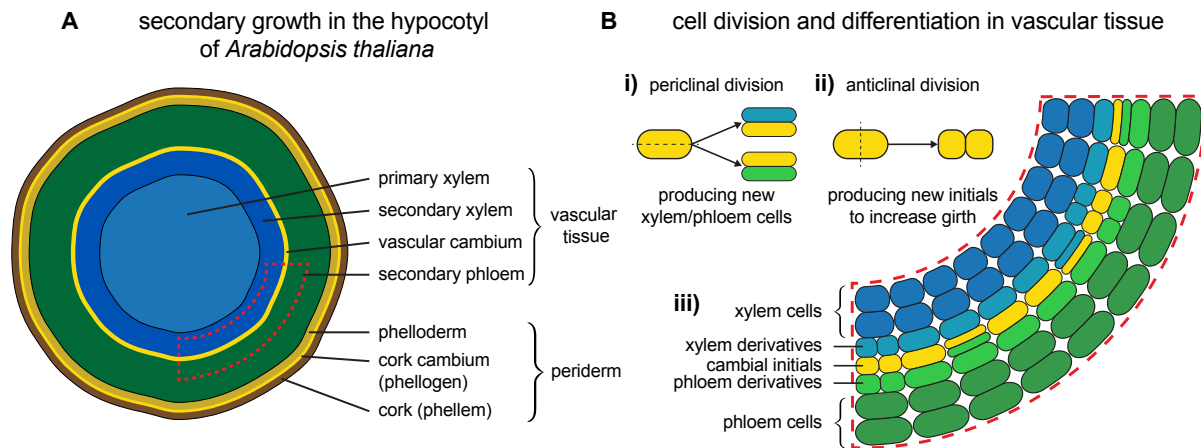


Figure 2.5: Secondary growth of the hypocotyl of *Arabidopsis thaliana* and cell division and differentiation in the vascular cambium: (A) During secondary growth pericycle-derived phellogen produces phellem outwards and phelloderm inwards, forming the periderm. This periderm surrounds the vascular tissue of primary and secondary xylem and secondary phloem. (B) Depiction of cell division and differentiation in the vascular cambium as the magnification of the highlighted area in (A). Cambial initials divide either periclinally to produce new xylem or phloem cells or anticlinally to produce new cambial initials that augment the plant’s capabilities for increasing its girth and producing more xylem and phloem daughter cells. Typically, those immediate cambium-derivatives divide one more time before fully differentiating¹¹⁵. Both figures are edited, and more detailed versions of the graphical abstract, published in¹³⁰.

stem. While the final structure of stems, roots, and hypocotyls look similar, the peculiarity of the hypocotyl lies within the somewhat strict separation of primary and secondary growth. Due to the absence of a developmental gradient along the organ length, elongation and secondary growth are effectively decoupled^{117,123}. Due to this possible distinction between the different growth modes, the hypocotyl of *Arabidopsis* is an advantageous model to study radial growth¹³⁰. Furthermore, during secondary growth, one may distinguish between different growth phases, the first one being characterized by equal production of xylem and phloem tissue (Figure 2.5B)^{32,56,117,125}. The beginning of flowering marks the transition towards the second phase, the xylem-expansion phase, where xylem fibers appear for further structural support of the tissue¹¹⁷. In this project, we consider only the first phase of secondary growth, where xylem and phloem are produced at equal rates, which experimentally was achieved by short-day conditions to delay flowering substantially¹³⁰. Note that at the early stages of secondary growth, due to these experimental growth conditions, the role of the periderm in the hypocotyl and its contribution to radial expansion can be omitted. This is because the periderm only starts to develop after about 3.5 weeks after germination. Because the samples we study are only about two weeks old, the periderm does not contribute to radial expansion, and only the vascular cambium drives secondary growth at this stage.

2.1.4 Plant cell division

As mentioned, plant cell division is primarily concentrated in the meristematic tissue. In the case of perennial angiosperms, this is the shoot apical meristem and root apical meristem for primary growth, and the vascular cambium and the cork cambium for secondary growth. These lay the basis for all increases in cell number and, thus, the growth and shaping of any plant organs. Division as a whole can be separated into three different phases: the interphase, mitosis, and cytokinesis. Each phase undergoes several steps¹² with the overall goal to duplicate the DNA and form a new cell wall (cell plate) to separate the two new daughter cells that emerge from one mother cell¹³¹.

The interphase can be considered a preparation stage of cell division and features three sub-stages. The first is the G1 (Gap 1) phase, where the cell grows and accumulates resources. Depending on the species and how fast tissue is growing, this stage can be either very short or take up to several weeks or years if the tissue is dormant¹² (cf. Appendix A) where cells only participate in growth and meristematic activity if environmental stimuli favor this^{99,104}. The subsequent phase is the S phase. At the G1/S checkpoint, the cell receives the signal to divide via intra- and intercellular signaling. The chromosomes are then duplicated, and the DNA is synthesized. After the S phase follows the G2 phase, where the cell grows further to prepare or directly transitions to mitosis.

Mitosis is the process where the cell progresses through several distinct stages to divide the nucleus and prepare for division, as illustrated in Figure 2.6. At either the end of the G2 phase or early in the so-called prophase (Figure 2.6A), cytoplasmic microtubules reorganize to form an array, the preprophase band encircling the pre-mitotic nucleus (see Figure 2.6A). Although this phase is brief, the preprophase band already determines the future placement of the cell wall that will ultimately separate the two daughter cells¹³¹. Thus, microtubules and their orientation play a vital role in division plane placement^{102,103}, which we will discuss more thoroughly in Section 2.2.3. In symmetrical cell division, the preprophase band is determined from the geometry of the mother cell. In contrast, in asymmetric cell divisions, an actin-dependent cell polarity seems to arise, exceeding shape-based influences¹³¹. To control the division plane orientation, the preprophase band promotes the formation of the mitotic spindle from microtubules^e (Figure 2.6B) in the direction perpendicular to the preprophase band. In addition, it assists cortical division sites to form that are left behind once the preprophase band is disassembled in the prophase and the mitotic spindle is formed¹³¹. Thus, it marks the future division site¹³². At this point, the nuclear envelope, previously surrounding

^eThe mitotic spindle functions differently in animals and plants as plants do not have centrosomes but use other proteins for spindle formation¹².

the nucleolus, has also vanished.

During metaphase, the microtubules of the mitotic spindle, together with motor proteins, push and pull the chromosomes to center them in the equatorial plane of the cell¹³³. Subsequently, in the anaphase, the sister chromatids get drawn towards the opposite ends of the cell (Figure 2.6C)¹². In the telophase, the nuclear envelopes re-form around the chromatids that start to de-condensate (Figure 2.6D). As a result, the cell is ready for separation by constructing a new cell wall.

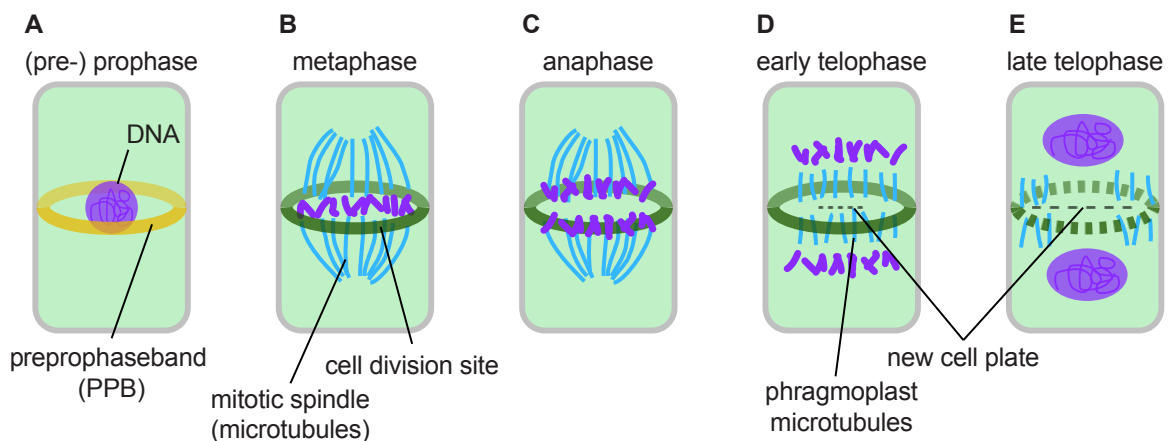


Figure 2.6: Phases of mitosis in plant cells (A) During the (pre-)prophase, the preprophase band is established from cytoplasmic microtubules and marks the cell division site. The nuclear envelope disintegrates, releasing the nucleus into the cytoplasm. (B) In the metaphase, the chromosomes are arranged in the equatorial plane of the cell. (C) In the anaphase, sister chromatids are pulled towards the opposing poles of the cell. (D) In the early telophase, the chromosomes have been separated. (E) At the late telophase, chromatids have de-condensated, and the nuclear envelope has been formed. Next, the cell plate is established during cytokineses, completing the division process.

During cytokinesis, the cell mother cell divides, and a new cell plate grows disc-like from the cell center towards the cell walls of the mother cells^{12,134}. The phragmoplast, a structure unique to plants, guides this process. It consists of aggregated microtubules, oriented in the direction perpendicular to the division plane¹³¹. Microtubules also guide vesicles of cell wall precursors from the Golgi apparatus to deposit at the new cell plate to contribute to the new wall and plasma membrane. Early in the process, callose is deposited for stability and supplemented by cellulose, the latter being the eventual main constituent of the newly formed primary cell wall. Once the newly growing cell wall has reached the existing walls of the mother cell, the phragmoplast depolymerizes, and the two new daughter cells are finally separated, except for permanent intercellular connections such as plasmodesmata¹².

2.2 Biophysical aspects of plant cells and tissues

After discussing cell and growth types and, in brief, the biology of plant cell divisions, we want to take a closer look into the physical principles behind these processes. For this purpose, we consider the mechanical forces acting during growth and division within individual plant cells, tissues, and organs. There are many ways in which physics affects plant morphogenesis across the scales from a cellular level to an organ level, for instance, mediated by internal pressure or external forces like gravity. To understand the physics of plant cells and tissues, it is instructive to first identify the physically most relevant components found within cells as the building blocks of larger entities (Figure 2.1). An individual plant can be considered an isolated, pressurized container in first approximation^{28,30,35}. In this picture, a tissue can then be represented by many pressurized containers embedded in a rigid network that allows mechanical interactions between them. However, the reality is much more complex, and the biophysics of plant cells comprises many aspects, including the precise regulation of mechanical properties, a response to internal and external cues and biochemical signaling, and controlling any changes. For the present context of radial growth or cell growth in general, the most important organelles are the cell walls as the load-bearing structures, the vacuole and plasma membrane that control the internal cell pressure to drive growth, and the cytoskeleton to control the directionality of growth^{15,16}. In the following, we will detail this subset of organelles and infer their influence on plant growth and mechanical forces, modifying individual cell shapes, tissues, and overall morphogenesis. In first approximation, these boil down to hydraulics as cell growth and transport mechanisms rely on control of internal pressure and yielding and growth of cell walls.

2.2.1 Turgor pressure

Turgor pressure is a plant cell's internal hydrostatic, isotropic pressure that results from water influx, driven by the osmotic potential¹⁰⁴. Turgor pressure is main driver of plant cell growth and like mechanical stress, it is measured as a force per unit surface. It is regulated by the vacuole (cf. Figure 2.1), which influences the cell's internal pressure, enabling active control of cellular expansion^{12,16}. Even though one might not immediately expect it, plant cells are under substantial internal pressure exerted by the internal components, the so-called protoplast, onto the cell's surrounding, rigid cell walls containing all organelles. Therefore, turgor pressure is the internal force that the cell walls, acting as load-bearing structures, must counterbalance. Such pressure typically ranges from 0.1 MPa to 1 MPa^{20f}. To understand the emergence of

^fFor comparison, an inflated rubber balloon has an internal pressure of about 5 kPa²⁰.

turgor pressure and distinguish between turgor and osmotic pressure, we must consider the water potential. In short, turgor pressure represents the hydrostatic pressure resulting from water uptake via osmotic potentials. The total water potential, denoted by $\Psi_W = \Psi_o + \Psi_p + \Psi_m + \Psi_g$, is the free energy of water per unit volume and determines the capability of a cell to take in water^{16,104,135}. It includes contributions from the osmotic potential Ψ_o , the pressure potential Ψ_p , the gravitational potential Ψ_g and the so-called matrix potential Ψ_m (Figure 2.7).

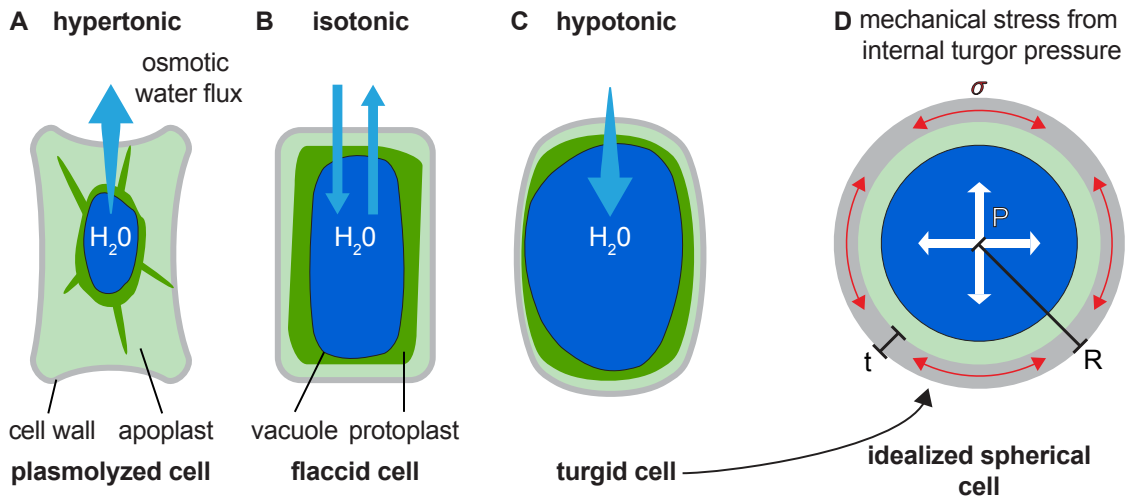


Figure 2.7: Schematic of individual plant cells with different tonicity and resulting pressure and mechanical forces. (A) In a hypertonic solution, a net flux of water reduces the internal pressure, and the cell deflates. (B) In an isotonic solution, there is a balance between the flux of water inside and outside of the cell. (C) In a hypotonic solution, there is a net inflow of water, resulting in an inflating, turgid cell that may build up enough internal (turgor) pressure to create tension, i.e., mechanical stress onto the cell walls to ultimately grow in size (cf. Section 2.2.2). (D) An idealized, spherical, turgid cell exerts uniform turgor pressure P onto its surface, creating uniform mechanical stress σ on its surface, i.e., the cell walls (cf. Section 2.2.4). R represents the radius and t the thickness of the cell wall.

The osmotic potential Ψ_o is a key factor for the water potential and water uptake of plants and reflects the concentration of solutes inside a cell¹⁰⁴. Pure water, by definition, has an osmotic potential of zero. For dilute solutions, the osmotic potential is given by $\Psi_o = -iCRT$ with the ionization constant i , solute concentration C , gas constant R , and temperature T . When solutes are added to water, the osmotic potential becomes negative as they reduce the water's free energy. Consequently, it is more likely that water will move towards areas with lower osmotic potential. Thus, it depends on the cell's surroundings if the osmotic potential contributes to an inflow or outflow of water to or from a cell. The different scenarios are displayed in Figure 2.7A-C. If the solute concentration outside of the cell is larger than on the inside, the osmotic potential outside is smaller, and water is drawn from the cell. In an isotonic solution, both the inside and outside have the same osmotic potential, leading to a net

balance in water flow. Finally, in a hypotonic solution, the osmotic potential inside the cell is lower than outside, leading to water intake, and the cell becomes turgid¹⁰⁴. This state is the one plants typically are in by accumulation of solutes and ions within them. The water uptake that this allows for is even required for the accumulation of high internal pressure, which is a necessity for cellular growth and expansion¹².

The pressure potential Ψ_p term represents the turgor pressure inflating a cell. It describes the pressure required to balance water entry into a cell and, thus, the difference between external and internal hydrostatic pressures that arise from the internal and external solute, i.e., osmotic potentials. Hence, the turgor pressure is $P = \Psi_{o(outside)} - \Psi_{o(inside)}$ ^{104,135}. The difference between the inside and outside of the cell depends on the amount of solutes in the water, which reduces the free energy and, with that, the potential.

Depending on the conditions, large increases in turgor pressure may occur, showing the requirement of a cell wall as a resistance to the forces building up to prevent the cell from bursting. In fact, turgor pressure creates substantial mechanical stress, acting on the cell walls, which depends on the shape of the cell, which we discuss in Section 2.2.4^{28,72,135}. The osmotic and pressure potentials far exceed the other two contributions in well-hydrated plants^{104,135}. Therefore, we will not consider them in more detail here. The gravitational potential Ψ_g , though, describes gravity's influence on water potential, i.e., acceleration, the potential energy of the water column, depending on its density. The matrix potential Ψ_m represents the potential from adhesive forces between water and solid surfaces like soil particles, mediated by hydrogen bonds, Van-der-Waals forces, and capillary action. The matrix potential thus depends especially on the surface area and is mostly relevant in dry growth conditions. Due to the chemical binding, water movement is restricted and has a higher impact in such settings, like in desert plants^{104,135}.

In summary, turgor pressure creates an internal pressure on the protoplast that the cell wall resists against. Thus, the internal pressure creates a mechanical, tensile stress on the wall¹⁶ (Figure 2.7C,D). The quantity and distribution of such mechanical stress depend on the turgor pressure and the cell's shape, respectively, as we will detail in Section 2.2.4.

2.2.2 Cell walls

Cell wall composition and structure

While cell biology and molecular signaling are important factors, plant cell walls are also fascinating structures from a physical perspective, as their organization and mechanical properties highly affect the overall growth of a plant cell. Cell walls surround the plant

cell's internal components, the protoplast^{12,16}. They serve as a cell's primary load-bearing structures, resisting the protoplast's substantial internal pressure, which is essential for driving growth. Additionally, they support the weight of the plant and can experience differential growth, potentially leading to competition between cells and tissues growing at varying rates¹⁵. Generally, we can distinguish between two distinct types of cell walls: the primary and the secondary. Primary cell walls define cell shape and provide structural support for the growing plant^{12,15,107}. They also play an *active* roll during growth by yielding to the internal pressure to elongate. Secondary cell walls, on the other hand, if present, are typically formed only after cessation of growth and provide a highly increased rigidity because they contain great amounts of the bio-polymer lignin. Secondary walls are found predominantly in sclerenchyma cells like tracheary elements and wood cells that are dead at maturity^{12,107}. Besides minor components like glycoproteins and enzymes (see Figure 2.8), cell walls are formed mainly of three different kinds of polysaccharides^{12,15}: The first type are crystalline nanofibrils, representing the stiffest type of polysaccharides. Typically, these are called cellulose microfibrils. The other constituents are the more flexible, complex matrix polysaccharides hemicellulose and pectin¹⁵. Depending on tissue type and species, they make up the major part of the dry mass of plant cell walls and occur in a ratio of 1:1:2 (cellulose, hemicellulose, and pectin)^{136,137}. Recently, traditional models of how these form cell wall structures have been challenged by computational modeling, allowing for a better understanding of the interactions between the three and their role in transmitting mechanical forces^{15,138}.

Individual microfibrils are elongated nano-rods of only 3 nm in diameter and 2 μm to 10 μm in length^{15,139}. Their crystalline structure results in a very high Young's modulus in comparison with the other cell wall materials of ≈ 100 GPa in axial direction^{15,140–142}. Cellulose polymers are synthesized directly at the plasma membrane by cellulose synthase enzymes, which assemble into multimeric complexes to form the cellulose structures. Cortical microtubules guide their site of synthesis, connecting mechanical forces directly with the deposition of cellulose microfibrils and, therefore, mechanical reinforcement of cells (see Section 2.2.3)^{15,100,101,139}. After polymerization, they aggregate to form layers of aligned bundles, surrounded by hemicellulose and pectins (or lignin in the case of secondary cell walls)^{12,15,139,143}. Each layer of microfibrils is differently oriented, contributing to directional differences in the cell wall properties (cf. Figure 2.8).

Hemicelluloses are a type of sugars and sugar residues that are synthesized in the Golgi apparatus (Figure 2.1) and transported towards the cell wall^{15,144}. In primary cell walls, xyloglucan is the most important agent to bind to the surface of cellulose microfibrils¹⁵ (cf. Figure 2.8).

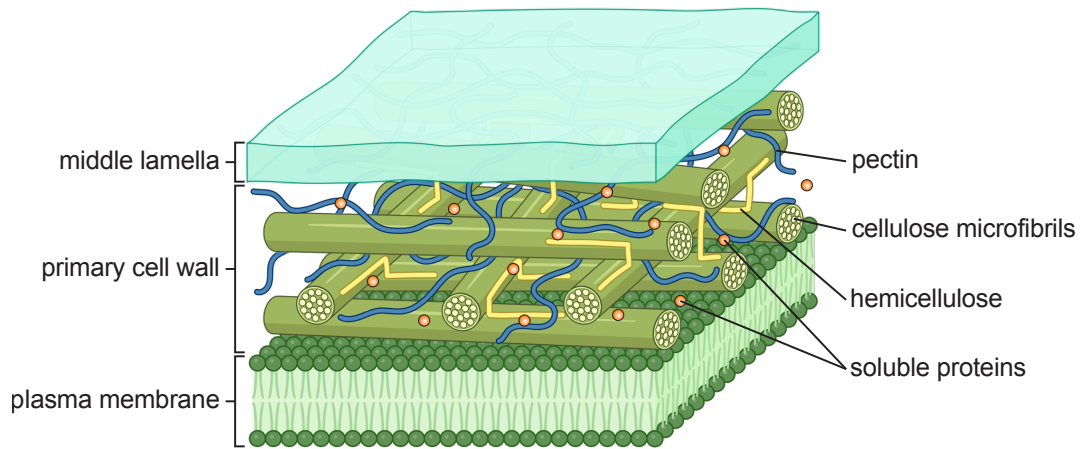


Figure 2.8: Schematic of a primary plant cell wall. The primary cell wall refers to the part between the plasma membrane and the middle lamella, the latter which adjoins individual cells to their neighbors by pectins. The main constituents of the primary cell wall are cellulose microfibrils, the matrix polysaccharides hemicellulose (especially xyloglucan), pectins, and soluble proteins. Towards the inside, the plasma membrane bounds the cell wall and encloses the protoplast. Created in BioRender. Höfler, M. (2024) <https://BioRender.com/w35u849>

While xyloglucan has been shown to influence the signaling of the growth hormone auxin, studies have revealed that xyloglucan has little impact on the direct wall mechanics and growth¹⁴⁵ and with ≈ 4 GPa also a much lower stiffness than cellulose microfibrils¹³⁸.

Pectins, besides their role as a stabilizer often found in food, are polysaccharides with a gel-like matrix between cellulose microfibrils (Figure 2.8) and are also produced in the Golgi apparatus. Pectins are the least rigid cell wall constituent, with an estimated Young's modulus of only ≈ 2 GPa. They have several purposes, such as hydration, porosity, ion exchange, and electrostatics, by which they influence the cell wall structure, thickness, and flexibility^{15,146–148}. They are present in the entire cell wall but especially so in the middle lamella (Figure 2.8), the outermost cell wall layer that fuses a plant cell with its neighbors^{15,149}.

Employing molecular dynamics simulations, Zhang et al.¹³⁸ have investigated the mechanisms of the wall assembly and mechanics based on the physical properties and dynamics of the just-described three main constituents of the cell wall. Matrix polysaccharides are not only less stiff in the axial direction, but they are also bending much easier, which is measured by their persistence length that is about 300 times smaller than the $3\text{ }\mu\text{m}$ persistence length of cellulose microfibrils^{138,150}. For this reason, cellulose microfibrils have the most significant impact on wall mechanics. Their arrangement, high stiffness, and persistence length provide substantial resistance to pull. Matrix polysaccharides, on the other hand, are typically entangled in the wall matrix and may coil up (see Figure 2.8). Thus, they rather uncoil instead of getting

stretched when pulled, providing comparably little mechanical resistance to any cell wall extension (cf. Figure 2.8). Different interactions arise that contribute to forming stable, coherent, and interconnected networks from the setup of cellulose, xyloglucan, and pectins as wall constituents. Amongst those interactions are direct cellulose-cellulose contacts, cellulose contacts with thin layers of water in between, xyloglucans binding to cellulose surfaces to act as an adhesive between them or potentially coiled-up xyloglucans between microfibrils. All of these possible interactions are embedded in the gel-like pectins^{15,138}. The impact of these interactions on the rigidity of the wall is hypothesized to decrease in the order mentioned. Understanding the relationship between bundles of stiff microfibrils and the less rigid matrix polysaccharides is crucial for understanding how mechanical forces influence cell wall growth and stress.

Considering the cell wall's rigidity naturally raises the question of how such an extremely stiff structure can still expand when a plant cell grows and how cell walls respond to mechanical stresses, which we discuss in the following.

Cell wall expansion

Cell wall growth is a continuous, cyclic process and is strongly coupled to the water uptake by the vacuole. This water uptake generates the internal turgor pressure to inflate the entire cell (cf. Figure 2.7C) and, thus, to induce mechanical stress on the cell wall (cf. Figure 2.7D)^{15,151,152}. The cell first responds by loosening the cell wall to alleviate mechanical stress. In this process, expansins, a group of proteins, are a key component alongside biochemical signaling, including the growth hormones auxin and brassinosteroids^{15,151}. Auxins play a central role as well, by acidifying the cell wall by activating proton pumps and lowering the pH to activate expansins and xyloglucan-specific enzymes. Expansins and enzymes then promote the yielding of the cell wall by breaking cross-linking hydrogen bonds between the cellulose microfibrils and hemicellulose. These processes make the entire network more extensible and allow cellulose microfibrils to reorient and slide relative to each other under tension. Brassinosteroids act in synergy with auxins, expansins, and enzymes by regulating the synthesis of the latter two and enhancing the auxin-signaling pathways to coordinate wall growth. The resulting sliding of microfibrils releases mechanical stress, and new cell wall material is synthesized^{15,153}. Due to the increased volume from the wall extension, turgor pressure decreases very briefly. A continuous water take-up counteracts this to restore turgor and re-establish the mechanical stress on the cell wall^{16,25}. By starting over with the entire process, sustained cell growth is achieved.

In mathematical modeling, the cell wall's irreversible growth and stress relaxation are often

described as a viscoelastoplastic process where cell walls irreversibly yield to the applied mechanical stress^{154–156}. The general idea, including the relationship between the stress-inducing turgor pressure and the mechanical resistance of the cell wall into a growth model, has already been proposed by James Lockhart in 1965¹⁵⁵. In his model, he considered the irreversible cell growth from cell wall yielding, which can be described in a simplified way by^{135,157}

$$\frac{dV}{dt} = \Phi(P - Y), \quad (2.1)$$

where Φ represents the extensibility of the cell wall, i.e., its mechanical rigidity, P is the turgor pressure, and Y is the yield threshold, which is the minimally required hydrostatic pressure to cause a yield of the cell walls. Thus, the critical parameter of the model is the yield threshold, allowing cellular growth only if pressure exceeds this threshold, effectively creating a balance between turgor pressure and yield. Consequently, plant cells are thereby considered similar to Bingham plastics¹⁵⁵, which are solid at low stresses but become liquid beyond a certain threshold, i.e., plant cells are considered to "flow" if the yield stress is exceeded.

More recent viscoelastic models apply a similar understanding of the processes, illustrated in Figure 2.9, resembling a Maxwell model of springs and dashpots^{15,158–160}. Here, the cell wall is also considered elastic to some extent but may also irreversibly creep if sufficient force is applied. The initial configuration is an elastic wall under high stress due to turgor pressure (Figure 2.9A). Expansins, auxins, and brassinosteroids mediate cell wall loosening, allowing the walls, i.e., cellulose microfibrils, to slide irreversibly. Consequently, the springs contract and tension is reduced (Figure 2.9B). Subsequent water influx restores stress, and the springs, i.e., walls, are stretched again (Figure 2.9C). This cycle then continuously repeats.

From the aforementioned concepts for cell wall expansion, we may already learn that mechanical stresses are a considerable factor in cellular growth. The process of cell wall loosening and the involvement of expansins, auxins, and brassinosteroids furthermore hints at the necessity of mechanisms for plant cells to sense stress and thus direct and redirect biochemical signals to efficiently guide the process. Unfortunately, a further consideration of biochemistry in this context is beyond the scope of this work. In the following section, though, we will address nonetheless the question of stress sensing regarding cortical microtubules, which, as we will see, play a crucial role in plant tissue development.

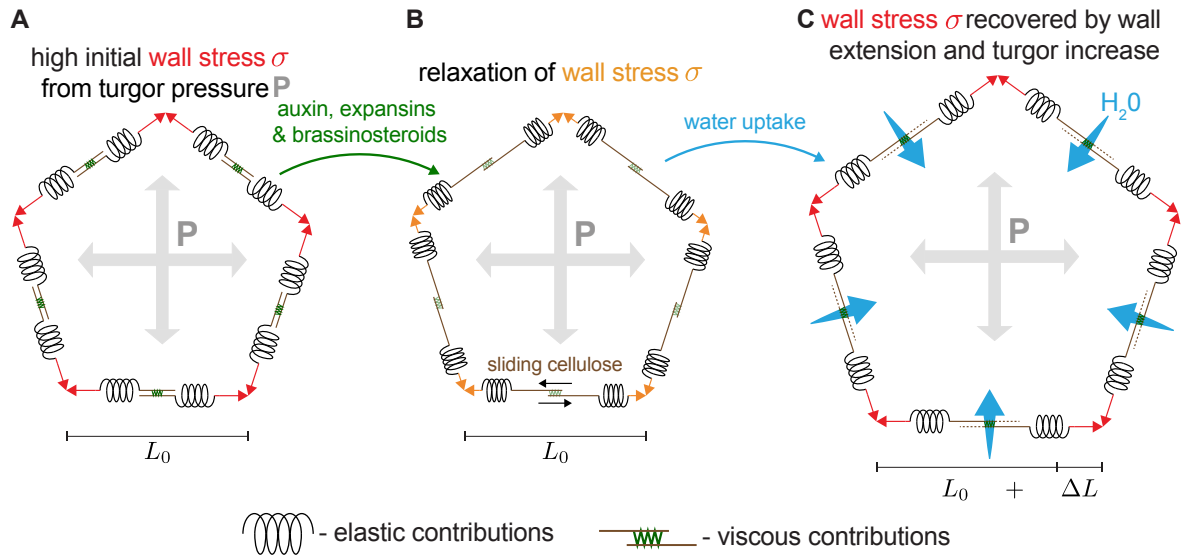


Figure 2.9: Stress relaxation of the cell wall represented by a visco-elastic model. (A) Initially, mechanical stress σ , depicted as extended springs with red arrows, is imposed on the cell walls due to turgor pressure P . (B) Upon cell wall loosening, mediated by auxin, expansins, and brassinosteroids, cellulose microfibrils may slide irreversibly in response to the high turgor pressure stretching the walls, which relaxes the stress σ on the walls (yellow arrows), represented by the springs contracting. (C) The cell size increases from its initial length L_0 to the new length $L_0 + \Delta L$ due to creeping of the cell wall. Turgor pressure P and, with that stress σ , is almost immediately recovered via water uptake, enabling further growth.

2.2.3 Cortical microtubules as stress sensors of plant cells

Cortical microtubules represent an integral component of cells and are involved in many cellular processes, most notably cell division and guiding the synthesis of cellulose^{100,101,131}. They constitute one component of a cell's cytoskeleton, with actin filaments forming the other one¹². Microtubules consist of the protein tubulin, which forms polymers from α and β -tubulin dimers to create a thin, hollow spiral of about 25 nm in diameter (Figure 2.10A, top). They are dominantly synthesized at the plasma membrane, and three orders of magnitudes stiffer than actin¹⁶¹. In consequence, they have an exceptionally high persistence length of about 4 mm to 8 mm and are highly capable of retaining their shape and any anisotropies due to their high bending stiffness^{162,163}. Despite being so thin, they reach typical lengths comparable to cell diameters of approximately 15 μm ^{164,165}. Usually, microtubules grow rapidly from the β -tubulin end, the + end (plus end), while growth at the α -tubulin end, or – end (minus end), is much slower. The – end typically remains stable, especially when anchored to the plasma membrane. It may also grow if it is not anchored to the plasma membrane. However, microtubule growth is not continuous; it involves a constant alternation between polymerization (tubulin addition) and depolymerization (tubulin loss). This behavior, known as dynamic instability, allows microtubules to rapidly reorganize in response to cellular

needs¹⁶⁶. Thus, there is a balance between the different growing, stagnating, or shrinking scenarios. In addition to this dynamic instability, also tread-milling behavior is observed, meaning a dynamic unidirectional (de-)polymerization, allowing for dynamic and sustained motility of microtubules^{166,167}. While about 90% of these tread-milling microtubules are very short in length, about 0.5 μm to 2 μm , they were shown by experiments of microtubule-severing protein katanin to have a significant impact in the global cortical microtubule organization^{67,168}.

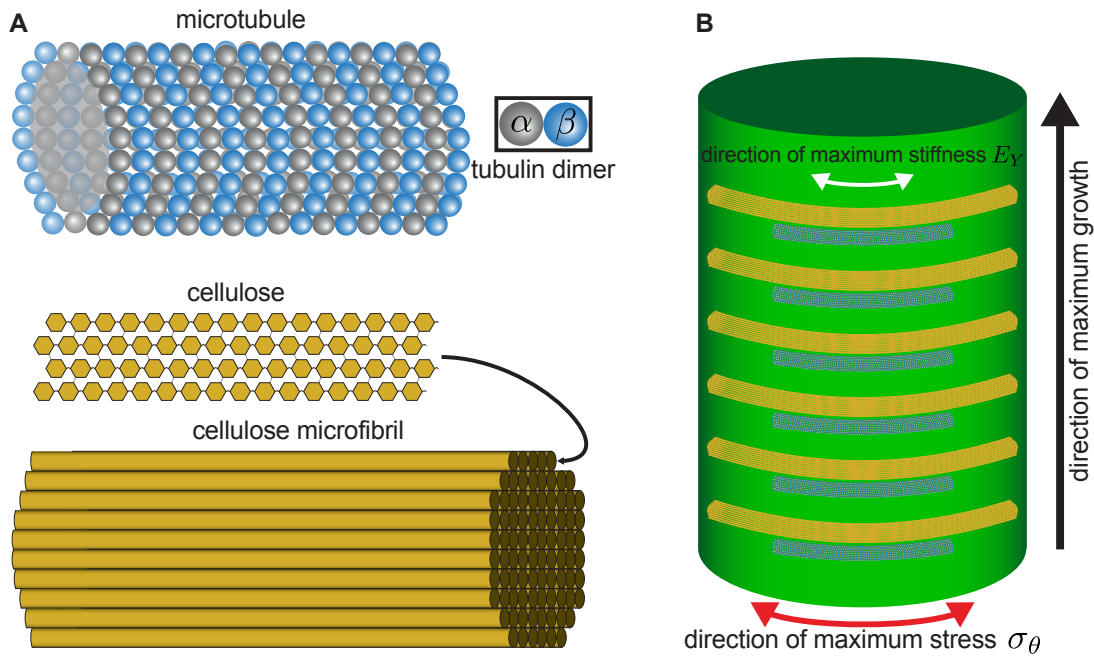


Figure 2.10: Microtubules guide deposition of cellulose microfibrils in response to mechanical stimuli. (A) Structure of cortical microtubules and cellulose microfibrils. Microtubules are built from tubulin dimers arranged in a helical, hollow tube (top). Cellulose microfibrils consist of polymer chains of cellulose connected by hydrogen bonds and Van-der-Waals forces (bottom). (B) Microtubules align with the direction of maximum stress on the cell wall (red arrow), leading to a directed deposition of cellulose microfibrils, ultimately anisotropically changing the stiffness of the cell wall, comparable to hoops around a barrel. Consequently, the direction of maximum growth is typically perpendicular to the direction of mechanical reinforcement. Panel (B) adapted from¹³⁰.

In Section 2.1.4, we discussed the basics of cell division from a biological perspective. As mentioned, cytoplasmic microtubules are critical for the division wall placement. In addition to this, from the previous section, we could also conclude that cortical microtubules guide the deposition of cellulose microfibrils (Figure 2.10A, bottom) by which the mechanical properties of the cell wall and thus the mechanical behavior of the entire cell changes^{15,100,101,139,143}. We already know that microtubules are closely linked to division plane placement and cellulose deposition. However, if microtubules control or guide these processes, the key question is:

What guides the microtubules themselves? In particular, we want to pick up the question that Hamant et al.¹⁸ raise if microtubules are tension sensors and if their alignment might result from a cell's mechanical state.

To be precise, we are concerned here with cortical microtubules. These microtubules localize at the cell cortex⁸. The first clues towards stress sensing come from observing the impact of mechanical forces on the dynamic instability of microtubules. Recent research suggests a connection between tension in the lattice and the occurrence of specific tubulin configurations. These configurations are then selectively targeted by microtubule-associated proteins (MAPs), thereby providing even long-range feedback between mechanical forces and MAPs to regulate the growth and shrinking of microtubules^{169–171}. Such feedback suggests a stabilization of microtubules under tension and destabilizing under compression, thus highlighting the role of mechanics in their orientation and self-organization¹⁸. *In silico* research has also shown that microtubules that are grown in a three-dimensional container will self-organize and localize at the boundary, i.e., the cortex of a cell, making them become cortical microtubules by nature of their mechanical properties²¹. These were found to typically align towards the long axis of a given cell, minimizing their bending energy and thus showing their ability to sense geometry and detect the longest axis of a cell^{21,22}. However, in plant tissues, this bias towards longitudinal alignment, i.e., the influence of geometry on the microtubule alignment, can easily be dominated by superimposing additional tissue- and growth-derived mechanical cues^{23,69}. Thus, geometry is one factor of microtubule orientation, yet a potential competition with additional cues might arise, which will be discussed in more detail in the next Section 2.2.4. While this shows that it is not simply geometry alone that guides the orientation and organization of microtubules, the study of Colin et al.⁶⁹ along several other^{23–25} provide evidence that microtubules do react in response to mechanical stress in plant cells. Experimental evidence indicates sensing and self-organization to be based on rapid changes in mechanical forces^{18,165}. Nevertheless, it is unclear if the stress pattern at a static state can also be perceived, which might explain the disparity of microtubule alignment in faster and slower-growing tissues⁷⁰. A key question regarding the dynamics of this context is if this tension is directly transmitted from the cell walls as load-bearing structures and sensed by the microtubules. As mentioned, cortical microtubules are localized, synthesized, and anchored at the cell membrane¹⁰³. The true nature of the cell wall plasma membrane-cortical microtubule continuum remains, however, a mystery¹⁷². Previously, microtubules were observed to

⁸Note that the cell cortex, the cytoplasmic layer beneath the cell membrane, should not be confused with the cortical cells of organs like the hypocotyl, stems, or roots (see Sections A.1 and subsequent sections), where the cortex represents a tissue layer between the epidermis and vascular tissue.

anchor to the plasma membrane; yet, this process requires specific anchoring points¹⁸. While plasmodesmata serve this purpose, they are relatively few in number and thus need further contributors. Possible explanations involve proteins to bind and bundle microtubules¹⁰³, retaining some degree of freedom for cortical microtubules to (re-)orient while anchoring to plasmodesmata, the cell-to-cell connections. In addition, proposed factors for this could be binding forces between cortical microtubules and cellulose synthase complexes¹⁷³.

In addition to the question of sensing, another aspect to be explored is the distinction between stress and strain. These two may differ substantially in different directions in anisotropic materials like cell walls. In plant cells, due to the anisotropy of cellulose microfibrils in their walls, the direction of stress is often perpendicular to the direction of strain (Figure 2.10B). Also, the transmission of tension would highly depend on the anisotropy of the cell wall¹⁸. This dependence on anisotropic cell wall properties might be the key to making the desired distinction between strain and stress. Suppose cellulose microfibrils align in a particular direction. In that case, growth will be maximal in the perpendicular direction (Figure 2.10B), meaning that also the cell wall will be more homogeneous in this direction¹⁸. Wall heterogeneities might also lead to strain discontinuities that destabilize microtubules in that direction, allowing for an indirect alignment mechanism by increased depolymerization. Another aspect appears when considering the proceeding growth on a longer timescale. From the reduction of mechanical stress over time due to cellulose deposition, the direction of maximum tension might also turn towards the previously minimal direction of stress, i.e., the direction of maximum strain, or result in a less extreme disparity between the two. This orientation difference between strain and stress could also explain why microtubule orientation changes over time in several contexts when transitioning from fast elongation to slower growth or even cessation, suggesting the possibility of dynamic changes between the direction of maximum strain and stress^{37,70}. As such, the robustness of growth and shape during plant morphogenesis could result from an automatic response of microtubules to mechanical stress, providing a dynamic loop of mechanical feedback to growth and cell wall remodeling¹⁸.

No matter the precise mechanism behind it, mechanical forces have been repeatedly shown to align with the direction of maximal stress¹⁸. This includes protoplasts⁶⁹, hypocotyls^{25,60,70}, shoot apical meristems²³, leaves⁷¹ or stems⁷⁰. The same is observed on a multi-cellular scale²³ with different means of imposing mechanical stress, for instance, by stretching^{25,60}, compression^{23,60,71,174}, ablation²³ or drugs⁶⁷. There are a few exceptions, like young hypocotyls undergoing primary growth where microtubules first rotate but stabilize after elongation growth ceases¹⁷⁵.

From all these observations, it seems highly convincing that microtubules are indeed tension

sensors, which will also be the premise for the present study. As microtubules are closely linked to cell division, one might wonder whether there is also a connection between mechanical stress and the division plane orientation via microtubule orientation. In the case of the shoot apical meristem, indeed, the direction of mechanical stress has even already been linked to cell division¹⁷⁶. Similarly, Hoermayer et al.¹⁷⁷ showed that spontaneous changes in mechanical stresses via wounding may alter both microtubule and division orientation. Thus, this leads us to the central question of this study: If microtubules are indeed tension sensors and play a big part in both the mechanical reinforcement of cells and the cell division orientation, is this also true for the radial growth of the hypocotyl and its bi-facial stem cell divisions in the vascular cambium and how do they affect the division wall orientation? We seek to contribute to the answer to this question in Chapter 5.

While we did now consider where anisotropies in individual cell walls arise, we did not yet give the overall cell and tissue dynamics much consideration. Hence, we will look into this next before proceeding with the formulation of our mechanical model for *in silico* analyses of tissue development in the hypocotyl.

2.2.4 Plant growth and mechanical stress on a cell and tissue level

In the previous sections, we thoroughly considered the biological background of plant cells and cell walls. Here, we want to discuss the impact of mechanical forces on plant cells and tissues as a whole based on the two most significant components: the turgor pressure and the cell walls. These aspects are, however, properties on a cellular scale. Beyond the individual cellular expansion, it is also important to consider that tissues, as networks of interconnected, inflated cells, form a rigid structure with shared cell walls. Therefore, we must also account for how neighboring cells respond to mechanical cues and how these cues propagate throughout the tissue, which we discuss in the following.

In essence, growth is driven by turgor pressure. Growing cells inflate by water intake to increase internal pressure, which puts mechanical stress on the cell walls. Beyond a particular threshold, cell walls yield irreversibly to the tensile stress, demonstrating the immense internal pressure acting on the walls. If we abstract a cell as a pressurized, spherical shell (Figure 2.11A), assuming thin walls, we may describe the mechanical stress acting on it by¹⁶⁰:

$$\sigma = \frac{PR}{2t} \quad (2.2)$$

where P is the internal pressure, the turgor pressure, R is the sphere's radius, and t is the thickness of the shell, i.e., the thickness of the cell wall. This stress acts isotropically

on the walls of the sphere. Because plant cells, especially cambium stem cells and their derivatives, are typically not round but highly elongated, one common approximation of the mechanical forces on plant cells is to consider a pressurized, cylindrical shell instead of a sphere (Figure 2.11B). In typical plant cells, for example in *Arabidopsis*, the thickness of the primary cell wall is about 50 nm to 1000 nm^{h31}, the turgor pressure between 0.1 MPa to 1 MPa²⁰ and typical radial cell sizes around 5 μm ¹⁵¹. Thus, the cell wall can be safely considered thin compared to its diameter. Longitudinal dimensions are often multiples of their diameter. In the case of vascular cambium, the elongation easily reaches tenfold the radius or even more^{12,114}. Using the, therefore, valid assumption of thin walls, we find that a particular stress anisotropy may already arise purely from the shape of an object. Specifically, for a static, isolated cylinder, the mechanical stress in the circumferential direction σ_θ is twice as large as the axial stress σ_z . This relationship can be approximated by:

$$\sigma_\theta = \frac{PR}{t}, \quad (2.3)$$

$$\sigma_z = \frac{PR}{2t} = \frac{1}{2}\sigma_\theta, \quad (2.4)$$

$$\sigma_r = -P, \quad (2.5)$$

using the same variables as before. The radial stress σ_r remains isotropic because it depends solely on the turgor pressure P . However, due to the thin wall approximation, this radial component has only a minor influence on the overall stress distribution within the cell. While this is a reasonable approximation for isolated, uniform cells, more complex shapes lead to more complex stress patterns, which require considering both the origin of such shapes, as well as their effect on tissue mechanics^{72,178,179}. This is also because even though the forces generated by internal turgor pressure are inherently isotropic, the same is not necessarily valid for the cell's response to these forces. Due to cell shape and inhomogeneities in the cell wall thickness and thus mechanical properties of the walls, even a single cell may be subject to a highly variable stress pattern¹⁵. Furthermore, considering the distribution of cellulose microfibrils along the walls of the cells, additional effects come into play: Since cortical microtubules generally guide cellulose deposition and tend to self-organize, their energetically preferred configuration aligns with the long axis. Essentially, this behavior arises from their tendency to minimize bending energy, which favors alignment along the long axis as this requires less bending overall. However, this preference for longitudinal alignment is weak and can be overridden by additional directional mechanical cues²¹. Nevertheless, due

^hThe upper end of this range is observed for example for epidermal cells, requiring especially high stability. The internal cells considered here typically have much thinner walls.

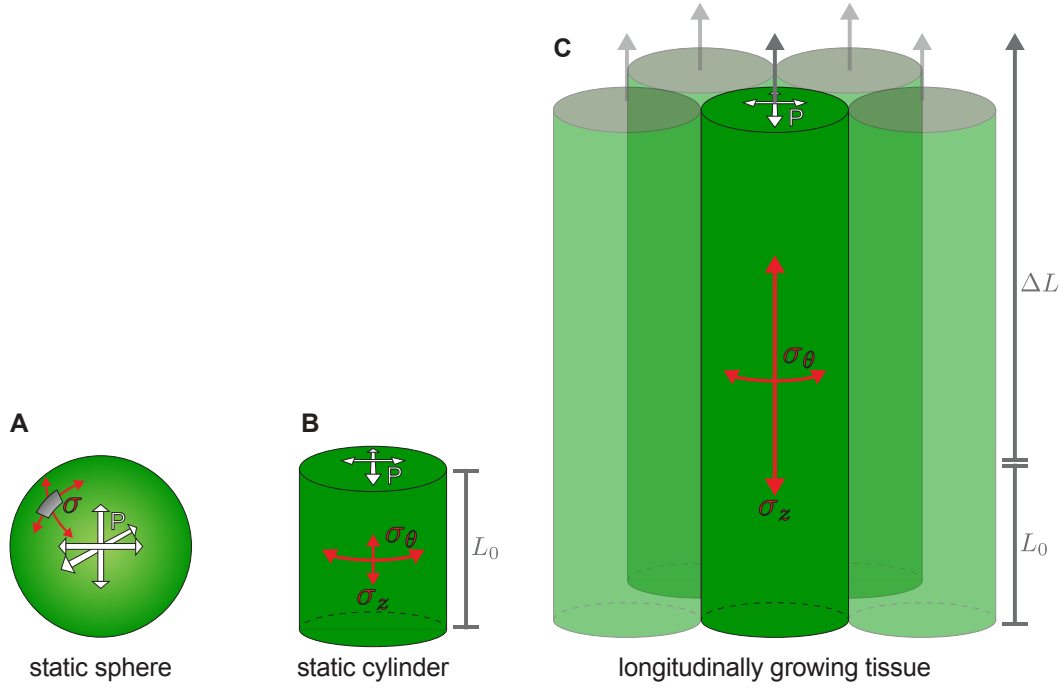


Figure 2.11: Mechanical stress from internal pressure depends on the geometry of the inflated object. (A) On a spherical surface, mechanical stress σ is uniform on its surface. (B) For a pressurized cylinder, mechanical stress σ_θ in circumferential direction is twice as large as stress σ_z in axial direction. (C) In an isotropic, homogeneous, linearly elastic material, creating an axial strain to cause elongation of a cylinder may lead to axial stress σ_z increasing, possibly exceeding circumferential stress σ_θ . Beyond that, additional mechanical cues from the surrounding, interconnected cylinders (or cells *in vivo*) may apply if not only considering isolated cylinders as an approximation for individual cells (not shown). Figure adapted from¹³⁰.

to this dependence, the properties of the cell wall will inevitably vary spatially due to the non-uniform distribution of microfibril density. If these microfibrils bear the load on the cell wall, they introduce anisotropies in the mechanical properties and responses based on stress. They are thereby altering the relationship between transverse and lateral mechanical stresses via anisotropic mechanical reinforcement^{15,30}. For instance, if considering again the stress pattern of the isolated cell (cf. Figure 2.11B) and then assuming a threefold density of cellulose in circumferential direction as opposed to the axial direction, i.e. the number n of cellulose microfibrils $n_\theta = 3n_z$, we would get a ratio^{15,30}

$$\sigma_\theta = 2\sigma_z \left(\frac{n_z}{n_\theta} \right) \quad (2.6)$$

$$= \frac{2}{3}\sigma_z, \quad \text{for } n_\theta/n_z = 3. \quad (2.7)$$

Thus, axial stress in such a case even dominates over circumferential stress due to the material anisotropy, promoting axial expansion.

Beyond a consideration as an individual cell, it is also vital to acknowledge both the effect of the surrounding tissue and its dynamics (Figure 2.11C). As described earlier, plant cells provide a rigid cellular array through the cell walls connecting them. Thus, changes in one cell may affect its neighbors and beyond, which plants exploit during morphogenesis to guide tissue and organ development^{26,39}. In this context, Kennaway et al.¹⁸⁰ distinguished between specified growth and effective growth. As specified growth, they described an expansion that would be observed without opposing mechanical constraints. In contrast, resultant growth refers to the effective, observed growth, including intercellular factors and constraints. Suppose some region within a tissue grows faster than the rest. A mismatch between the strains and the stresses across different organ parts will occur in this case. Such a mismatch may lead to mechanical conflicts because of residual stresses accumulating over time. Ultimately, these stresses hold the potential of giving rise to non-uniform expansion and shape due to the anisotropy of the forces exerted²⁶ (cf. also Figure 3.1). We will also discuss examples of non-uniform expansion and its modeling in Chapter 5. Overall, mechanical conflicts between different tissues are widespread and affect or even enable the creation of complex shapes in the first place. Examples include the formation of leaves^{26,39} or flowers^{39,40}. This highlights again the necessity of the distinction between the growth of individual cells on the one hand and tissues as an ensemble of cells on the other hand, also regarding the resulting stress patterning¹⁸¹.

Such considerations have already been made by Hejnowicz et al. in the 1990s^{25,182,183} and later picked up in more recent research for the examples of *Arabidopsis thaliana* and *Helianthus* (sunflower) hypocotyls^{60,70}. In their work, Hejnowicz et al. proposed a superposition of primary cellular stress, which is a function of individual cell shape, and secondary tissue stress as the resulting tension from the entire tissue structure²⁵. By measuring Poisson's ratios for the uniaxial and multiaxial application of strain to sunflower hypocotyls, they computed the mechanical stresses in longitudinal and transverse directions, realizing that if both shape-derived and tissue-derived stresses are superimposed, axial stress may dominate in such a system. This result was approved in later research on *Arabidopsis* hypocotyls, where it was shown that the direction of maximum stress also depends on tissue constraints, especially in the case of the epidermis. Here, Robinson et al.⁶⁰ demonstrated that the epidermis effectively restricts the hypocotyl elongation due to its thick cell walls, compressing the internal tissue that would otherwise expand longitudinally. Also, in this case, mechanical stress was shown to be in the longitudinal direction, even though in isolated cells, one would expect mechanical stress to dominate in the transverse direction, considering the model of a thin-walled pressurized cylinder. Another independent work by Verger et al.⁷⁰ drew the same

conclusion and pointed out the importance of growth dynamics to the prevalent stress pattern. They showed that in the case of the fast-elongating hypocotyl of *Arabidopsis*, growth-derived tissue stresses dominated over shape-derived stresses. In contrast, shape-derived stresses seemed to be the driving factors for slow-growing tissues because of relaxation processes. Ultimately, mechanical conflicts between static and dynamic effects, i.e., primary and secondary contributions to the global stress patterns, seem to arise during elongation growth. This observation is consistent with findings in the context of leaves where cortical microtubules detached from the plasma membrane to align based on the cell geometry after elongation stopped³⁷.

To summarize, if considering the complete picture of plant tissue growth and morphogenesis from a cellular to an organ scale, many different factors have to be taken into account: Cell shape and shape variations, mechanical properties of individual cells, and changes among various cell types, growth dynamics, i.e., growth speed and pattern, and external stimuli. The latter also includes stimuli like gravity or light, determining growth dynamics to a large extent via biochemical signaling, again influencing the mechanical properties of cells¹²². In addition to their individual contribution, all of the previously mentioned interact with each other to some degree, making a decoupling of different processes highly challenging. Figure 2.12 shows an overview of the arguably most important interactions between mechanical (blue) and biochemical influences (green). These different aspects, in combination, provide a profound feedback loop of all the intra- and intercellular processes that are linked during plant tissue growth and morphogenesis. Consequently, considering the entirety of details to this end seems almost impossible; hence, for biomechanical modeling, we always have to judge, ideally based on experimental evidence, which key factors must be included to produce meaningful and convincing results. This work focuses on the mechanical interactions during cell and tissue growth, considering in particular how cell division may fit in this picture and its connection to mechanical stress (Section 5).

Based on the findings from previous research presented in this section, we draw two main conclusions: First, we can reasonably assume that the maximum stress in three dimensions may occur in the longitudinal direction of the hypocotyl, i.e., the direction of organ elongation. This is highlighted in our analysis of the radial growth of the hypocotyl of *Arabidopsis thaliana* in Chapter 5, where we assume that longitudinal stress predominates, suggesting a hypothesized connection between mechanical forces and tissue development, including cell divisions. As discussed in Section 3.3 and Chapter 5, a significant challenge is the frequent lack of reliable data for physical parameters such as Young's modulus. This issue

results from the considerable variation in these parameters across different cell types, even within the same organ or tissue, and changes over time due to biochemical signaling and growth processes. Therefore, our second conclusion is that we must minimize the number of parameters we use until advancements in measuring and imaging techniques provide us with further information^{65,184}. With these assumptions and fundamental insights into the growth and development of plant tissues, we now proceed with the theoretical description of our modeling approach, formulating a mechanical model to study the growth and development of the hypocotyl *in silico*.

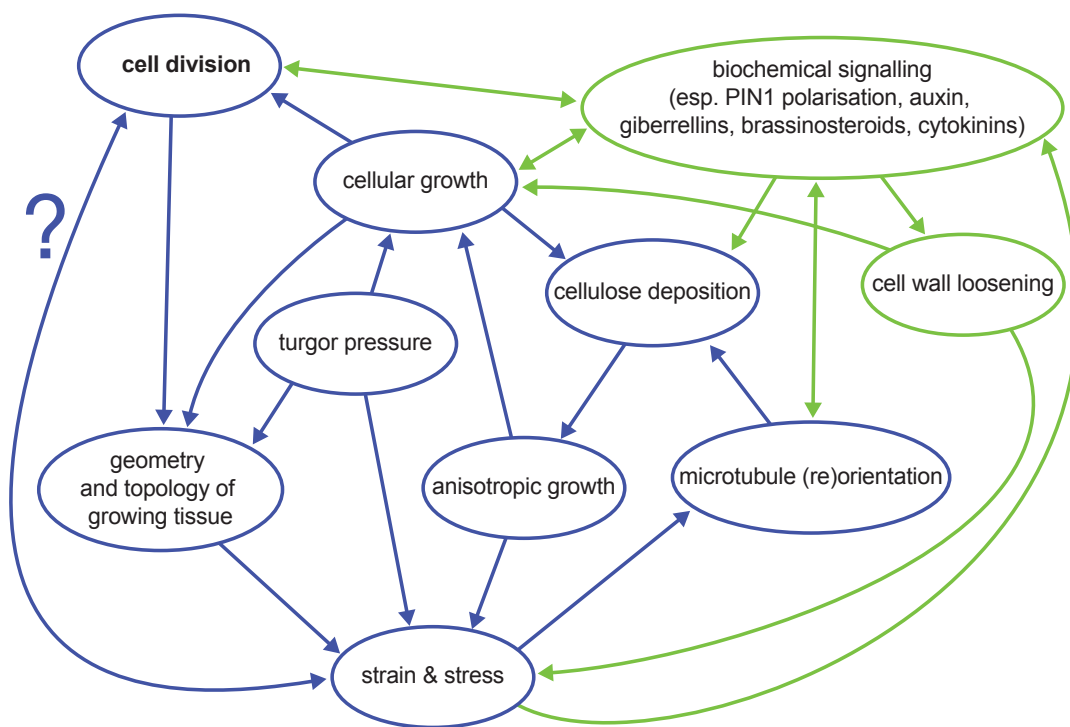
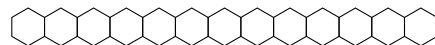


Figure 2.12: Summary of the connections between mechanical (blue) and biochemical (green) processes. The combined abundance of intra- and intercellular processes and their connections lead to a profound feedback loop between cell and tissue mechanics and biochemical signaling. In particular, we are interested in the role of cell division concerning cell mechanics and possible connections between the two.

3

Mechanical modeling of plant tissue development

This chapter explores the mechanical modeling of plant cells. We first introduce different mechanical modeling frameworks, discussing their applications, advantages, and challenges. Next, we present our cell-based vertex model, starting with conceptual ideas and strategies for handling tissue topology and geometry using the quad-edge data structure. Then, we introduce the actual mechanical model we apply, describing the mathematical representation of cells and tissues through shape matrices^a as well as the applied biophysical principles. Overall, our focus is on establishing a framework for two-dimensional simulations that combine biological insights to create a robust mechanical model. In this endeavor, we lay out our approach to a mathematical description of the biophysical mechanisms of growth and mechanical feedback mechanisms between directional growth and mechanical stress. We also discuss topological changes, such as cell divisions and different division rules. Finally, we provide an overview of the implementation of our simulations and the overall workflow behind them.



3.1 Modeling overview

When thinking about the mechanical modeling of plant tissue and its morphogenesis, the natural question that arises is how to implement a biological process in a simulation and which framework to use. For this decision, it is instructive to consider the desired purpose,

^aNote from this chapter onward, we typically apply the terms "cells" and "tissues" given the biological background of this work. In contrast, we refer to "polygons" and "(polygonal) meshes" when focusing on geometrical and topological considerations and properties, particularly in the respective theory sections.

accuracy, and associated requirements, constrained by the available computational power⁹⁰. These considerations also include scale and level of detail, for instance, whether one constructs a tissue or organ by utilizing a minimal amount of simple shapes such as triangles or hexagons or if the actual shape of cells and tissues needs to be closely matched to the real world. Regardless of the level of abstraction, detailed modeling of the mechanics requires an elaborate mathematical description¹⁵⁹. For this purpose, different types of models, each with a plurality of implementations, have been created. Here, we will briefly discuss the most common modeling approaches. From those, two major model types appear to be widespread in the community for their versatility⁹⁰: finite element models^{91,185} and Hamiltonian-based (vertex) models^{67,87}. We discuss further details, applications, advantages, and challenges for both models in the following. The general aim of such modeling is not only to provide descriptive approaches but also to consider the spontaneous creation of form. In this context, the resulting shape of a cell or tissue emerges from the model as a consequence of applying physical interactions and constraints, which may, for instance, lead to specific anisotropic growth or proliferation patterns¹⁸⁶. Therefore, instead of describing previously observed experimental findings, these models typically allow for predictions of emerging morphologies.

3.1.1 Finite elements models

Finite element models are computational models that allow for the mechanical modeling of large-scale systems of tissues or even organs by dividing the studied system into small, manageable components. These so-called finite elements allow for precise simulations of processes like growth or the response to internal and external mechanical cues^{186,187}. Due to the possibility of studying larger scale systems with potentially challenging geometry and topology, finite element models are a popular approach in plant science and are applied in various contexts of studying shape and growth^{26,46,85,86}. Originally developed to study aircraft structures¹⁸⁸, this model approach also finds wide application in other fields of engineering like civil engineering, heat conduction analyses, or mechanical design, to simulate the behavior of specific components and prototypes under load¹⁸⁹. Such applications also extend towards medicine to investigate bio-mechanics, e.g., the function of knee joints¹⁹⁰. The basic steps to set up a finite element model are the following¹⁷⁸:

- Define the application's geometry, dimension, and general shape (e.g., a two-dimensional system like a cross-section or a hemispherical dome when studying the shoot apex).
- Discretize the system into a chosen number of faces, i.e., a finite number of elements of vertices, connected by edges. Typically, this is achieved by either triangulation or

quadrilateral meshing. One might also add further individual mesh elements to the discretization to better capture the shape and, for instance, curvatures.

- Apply boundary conditions to account for external constraints such as rigid walls or fixing in place part of the system (e.g., the base of the shoot since it connects to the stem) and include any other interactions of the modeled organism with its surroundings, gravity, or global pressure.
- Define a material model for the mechanical behavior of the system, typically based on continuum mechanics.
- Construct a stiffness matrix^b for each finite element to compute the forces based on displacements of vertices and combine all elements into global matrices for the large-scale model.
- Infer desired quantities like stress and strain on a global scale from the displacement of nodes, boundary conditions, and other interactions.

In simulations of biological tissue, one may use artificial meshes or derivatives of experimental data. In the case of plants, one often considers an inflated thin shell or a solid body to model a plant organ^{23,60,68,86}. If considering a large-scale tissue, a trade-off between the accuracy of the meshing and computational efficiency may be necessary, especially if the biological cell size is much smaller than the resolution achieved by the individual elements of the finite element model. Therefore, if entire plant organs are simulated, an analysis of the effects of finite size may be required to determine the independence of the results on the discretization. Furthermore, a common way to exploit the strengths of each discretization type is by using an adaptive discretization, for instance, to better capture curvatures via additional elements and apply more coarse-graining where feasible¹⁷⁸.

From a mathematical perspective, in finite element models, one may decompose the total deformation gradient F in a composition of n steps of any pure growth G and any elastic tensor A , similar to Morris 2018^{186,187}:

$$F = A_n(G_n(A_{n-1}(G_{n-1}(\dots(A_1(G_1(\dots))))))). \quad (3.1)$$

The elastic tensor A_i can be computed at each simulation step. The growth tensor G_i can either be predefined to include anisotropies in growth or be computed using a patterning simulation

^bNote that the stiffness matrix in FEM modeling differs from the one known in continuum mechanics. Here, it refers to mapping a vertex displacement to a force with respect to an adjacent vertex.

or morphogen gradients. This allows for the consideration of contributions to the dynamics from auxins, cytokinins, gibberellins, or brassinosteroids. These biochemical agents alter the mechanical properties and influence the emergence of shape and form^{180,186}. For specified growth, one possibility is to infer the deformed state of elements from applying the growth tensor to the reference configuration of elements¹⁸⁰. Due to constraints of the surrounding tissue, residual stresses may arise, reflecting any spatial differences in the growth pattern. One could either take these residual stresses as they are, as input for cortical microtubules, cellulose microfibrils, and other mechano-sensitive cell components. Alternatively, these residual stresses may be relaxed by updating the reference configuration to the current configuration because of the assumption that the cell wall remodels/adapts to the current configuration, releasing these stresses over time. Any gradients introduced at the simulation's beginning will follow the tissue's corresponding material points during growth, allowing for the emergence of specific shapes¹⁸⁶. Thus, assigning fields to simulate specific growth properties allows us to determine a growth directional field (polarity) and a growth modulator, defining how a shape will grow over time, as qualitatively depicted in Figure 3.1. A popular example of a finite element modeling software, specifically for plant tissues, is *MorphoGraphX*⁹¹ with its derivative software *MorphoDynamX*¹⁸⁵.

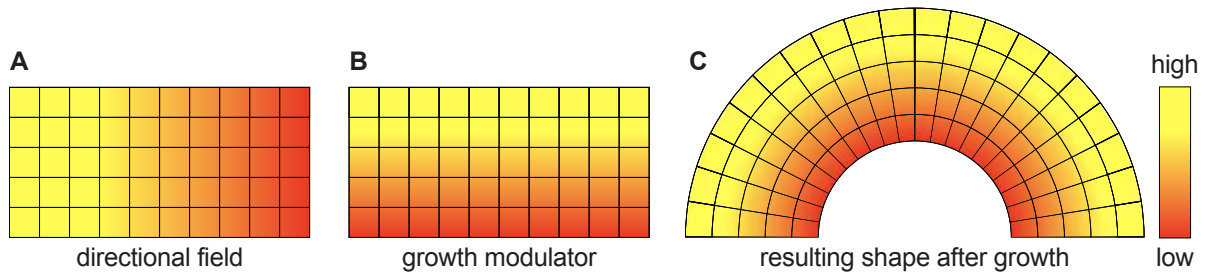


Figure 3.1: Growth, promoted according to applied morphogen gradients, guides anisotropic growth. Such a morphogen gradient may define a directional field (A) and modulate the speed of growth (B). In a case where both are exactly orthogonal, a tissue eventually grows to an arch shape (C).

3.1.2 Vertex models

As the name suggests, vertex models describe an approach using vertices connected by edges to form a polygonal network. The dynamics in such models are then captured and driven by mechanical forces to accommodate all phenomenological processes and physical variables. Using these contributions, a Hamiltonian is defined to describe the system's energy. Since this model is the one that the present work is based on, we will only give a brief idea of the

underlying concept here and discuss our implementation of the model in detail in Section 3.3.

The general concept behind vertex models is to construct a Hamiltonian based on the mismatch of a present configuration of a tissue, represented by vertex positions with respect to a target configuration. The quantity considered depends on the implementation, but typically, this results in the sum of the contribution of all cells, where its undeformed and deformed shape or area is considered for each cell. While other approaches are possible and have been applied in previous works^{57,67,68,88,89,176}, one possible implementation of a vertex model is the publicly available software VirtualLeaf⁸⁷. It allows for custom simulations of growing and dividing tissues based on a mechanistic approach where the instrumental quantities for the Hamiltonian $\mathcal{H}$ are a target area A_T and a target length L_T of wall segments, i.e.^{87,186}

$$\mathcal{H} = \lambda_A \sum_i (a(i) - A_T(i))^2 + \lambda_M \sum_j (l(j) - L_T(j))^2. \quad (3.2)$$

The summation in the above equation is over all cells i and cell walls j , where $a(i)$ is the area of cell i and $A_T(i)$ the target area. Furthermore, $l(j)$ is the cell wall segment length, and $L_T(j)$ is the respective target length. The coefficients λ_A and λ_M determine the magnitudes of the contribution of the individual terms^{87,186}. The general idea of such a model is to follow a quasi-static approach where, in each simulation step, an equilibrium configuration is sought, i.e., the configuration in which the total energy H is minimized¹⁹¹. This state of minimized energy corresponds to a force balance for all vertices involved. Different approaches are applied to find such a state. In the case of VirtualLeaf, a Metropolis algorithm¹⁹² is used where, iteratively, a random node is chosen to be displaced in a random direction by a chosen step size. Then, the resulting change in the total energy ΔH is computed, and the displacement is accepted if the energy change is negative. If positive, the change is still accepted with a probability, based on the Boltzmann distribution, i.e., $P = \exp(-\Delta H/T)$, where T represents noise, to avoid getting trapped in a local minimum of the potential landscape⁸⁷. Over time, to ensure continuous growth, the target area needs to be increased by a constant rate or a chosen growth equation. The most striking advantage of vertex models is that due to the energy optimization in every step, one may implement many cell behaviors by adding terms to the Hamiltonian. This highly flexible method allows us to reflect any desired physical or biological phenomenon. Depending on the precise formulation, the model is suited to simulate both plant and animal tissue. Particularly, simulations of epithelial tissue employing vertex models are popular, e.g., of *Drosophila*, *Xenopus* or *Hydra*^{193–195}. Regarding plant tissue, oftentimes organs like the shoot or roots of *Arabidopsis thaliana* have been studied by such an

approach^{43,57,67,68,88,89,176,196}.

In Section 3.2 and the following, we will detail the vertex model we have used in this work. Plant tissue modeling often uses phenomenological parameters that are not necessarily straightforward to tie to experimental measurements. In contrast, we connect our model with infinitesimal strain theory to link our simulations to real-world data. Infinitesimal strain theory allows us to include mechanical parameters, well known from standard continuum mechanics, such as Young's modulus and Poisson's ratio, that can be measured or estimated experimentally^{20,66,85}. In addition, we will incorporate further terms into the Hamiltonian to represent real-world phenomena such as turgor pressure and external mechanical cues.

3.1.3 Other models

Mass-Spring-Models

Mass-Spring-Models are an approach in which tissues can be subdivided into individual cells that are defined by connected springs as shown in Figure 3.2A. The springs connect mass particles, typically with uniform mass, forming a network. Retaining or simplifying the cellular structure from experimental data is especially advantageous since the springs, connecting vertices, can represent individual cell walls and outlines of cells. Because cell walls are the load-bearing structures of plant cells, they contribute the most to the structural integrity of the cell. In contrast, the internal compartments beneath the wall only contribute negligibly, highlighting the benefits of focusing on modeling the walls via springs¹⁸⁶. Similarly to vertex models, the system state of Mass-Spring-Models depends on the forces $\mathbf{F}$ acting on individual vertices v at position $\mathbf{x}$. Thus, typically, such a model may be described via the forces acting on each vertex as¹⁸⁶:

$$\mathbf{F}_v = \sum_{n \in N_v} k \left(\frac{\|\mathbf{x}_n - \mathbf{x}_v\|}{l_{v \rightarrow n}} - 1 \right) \frac{\mathbf{x}_n - \mathbf{x}_v}{\|\mathbf{x}_n - \mathbf{x}_v\|}, \quad (3.3)$$

with the neighbors N_v of vertex v , the spring constant k , the position of a neighbor vertex $\mathbf{x}_n$, and $l_{v \rightarrow n}$ the rest length of the spring between vertices v and n . The symbol $\|\dots\|$ denotes the Euclidean norm between vertices $\mathbf{x}_n$ and $\mathbf{x}_v$. From the terms in parentheses, if the rest length is greater than the distance between a vertex and its neighbor, the net force is positive, and if the distance is smaller, it is negative. The second fraction represents the normalization direction of the force. Beyond the direct interactions of springs, further forces, such as turgor pressure acting perpendicular to each cell wall (Figure 3.2A), can be included. Typically, a steady-state

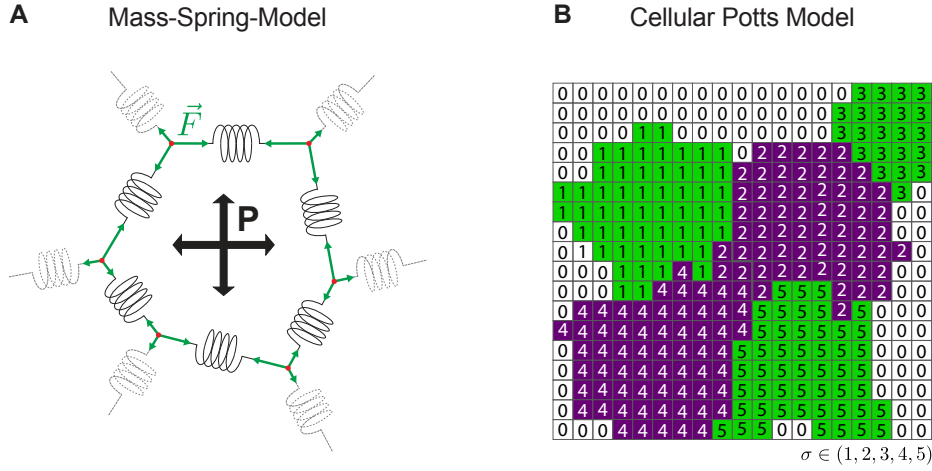


Figure 3.2: Mechanistic model approaches of different kinds. (A) Mass-Spring-Model for simulating growth by discretizing tissue and cells into a network of springs, connecting particle vertices (red dots). The net force $\vec{F}$ is calculated on each vertex. Additional forces, such as turgor pressure (P), can be included. (B) Cellular Potts Model for the discretization of the Euclidean space itself instead of the tissue or cell boundaries. Cells σ are marked by numbers and cell types by colors.

is sought for in these systems, i.e., the resulting system of equations is solved for $F_v = 0$, for instance, using a root-finding algorithm such as the Netwon-Raphson method¹⁹⁷,

$$x_{n+1} = x_n - \frac{f(x_n)}{f'(x_n)}, \quad (3.4)$$

where x_0 is an initial guess of the root of the function f , which can then be iteratively found. With this method, a system of equations for the velocity $\mathbf{c}$ and position $\mathbf{x}$ can be calculated from¹⁸⁶:

$$\frac{d\mathbf{c}_v}{dt} = (F_v - \zeta \mathbf{c}_v)/m, \quad (3.5)$$

$$\frac{d\mathbf{x}_v}{dt} = \mathbf{c}_v, \quad (3.6)$$

with a dampening constant ζ to avoid infinite oscillations and enable the system to converge into an equilibrium, and a mass m .

After finding the steady state, a growth step is performed by increasing the rest length of the spring to simulate wall yielding. One inherent problem with this modeling approach is that the model's outcome directly depends on the discretization¹⁸⁶. If one considers only one cell, a distortion of the cell in a direction parallel or perpendicular to the springs will have a very different effect. Distortions might not be an issue in round cells, with many springs representing cell walls. In longitudinal cross-sections of plant tissues like roots or stems, cells

are typically of highly anisotropic shape, possibly even rectangular. In such contexts, simple Spring-Mass models might be insufficient. Another potential issue is that residual stress might accumulate over time if adjusting the rest lengths does not match the actual stress build-up¹⁸⁶. For instance, such stress build-ups may be approached with the Lockhart growth formulation, possibly including a yield threshold to release such stresses¹⁵⁵.

Cellular Potts model

Unlike the previously discussed models, the cellular Potts model does not apply a discretization of the considered plant tissue or organ but rather the Euclidean space itself, which is displayed in Figure 3.2B. Like vertex models, it is also a Hamiltonian-based approach. Consequently, the cellular Potts model also has some similarities in describing cells and their state, where the cell area and boundary length or volume and tissue surface area are typically considered. Each cell σ corresponds to a set of labeled grid points to determine its type. The total energy H that is sought to be minimized can be typically written as a combination of either area or volume constraints and either boundary or surface constraints, reflecting the tendency of cells to grow and to adhere to neighboring cells¹⁸⁶:

$$H = \lambda_V \sum_{\sigma} (V_{\sigma} - V_{0,\sigma})^2 + \lambda_B \sum_i \sum_{j \in N_i} \tau(i, j), \quad (3.7)$$

with the current volume V_{σ} of a cell σ and $V_{0,\sigma}$ the target volume where the pre-factor λ_V represents the strength of this volume constraint in relation to the second term, adding a surface cost $\tau(i, j)$ of strength λ_B . The summation for the surface cost is for all neighbors $j \in N_i$ of a grid point i . Thus, the volume constraints are determined per cell, and the shared boundaries of all cells determine the surface constraints. As with vertex models, this provides flexibility by simply changing or introducing other terms to produce a specific behavior. For instance, a constant target volume or a size threshold can drive cell and tissue growth via cell division. Changing the boundary cost term might also introduce cell adhesion effects. Due to the significance of cell division and adhesion effects for many systems, topological transitions are often included^{186,198}. The basis for the dynamics in the model and the computation of energy changes is typically provided by a Metropolis-like algorithm^{192,198}, where the typical steps are as follows:

- Randomly choose a lattice site i .
- Choose a random neighbor $j \in N_i$ and change its label to the one of site i (cf. Figure 3.2B).

- Compute the difference ΔH for this change.
- Accept or reject the new configuration based on a Boltzmann distribution, i.e., accept if $\Delta H \leq 0$ and accept with a probability of $P = \exp(-\Delta H/T)$ if the change $\Delta H > 0$.

In the acceptance probability, T stands for the cell motility, which may be a function of the cell type if multiple types are considered. Due to the inherently stochastic nature of this rejection-acceptance method, cells, and cell walls or boundaries are ever-changing. Hence, beyond plant tissue simulations, it is also well-suited for animal tissue simulations because more frequent fluctuations occur. This may even be beneficial for the simulation of animal cells by their higher mobility, compared to plant cells, due to the absence of a cell wall that embeds them into a rigid network^{73,186}.

3.2 Topology and geometry in the cell-based vertex model

We now want to turn toward the cell-based vertex model we used for this study. First, we describe the framework behind topology and geometry in this model, which provides the scaffold for tissue modeling. For this undertaking, we need a spatial description of the tissue and individual cells, requiring to consider both the geometry and topology of the tissue. Additionally, we require a framework to describe dynamic processes, such as growth, cell divisions, or other topological changes.

3.2.1 Topology

The biological tissues we consider here are represented by polygonal meshes. Each polygon represents a single cell whose edges connect the surrounding vertices. By that, the entirety of individual polygons make up the geometrical description of a polygonal mesh. Yet a purely geometrical approach is insufficient, especially when considering changes in a mesh's morphology, for instance, as a result of cell division. Therefore, an efficient way of describing the mesh's topology is also required to allow access to the connections of each piece of the polygonal mesh, for example, to know which vertices and edges belong to a particular polygon. Leonidas Guibas and Jorge Stolfi proposed an exquisite approach to this in a brilliant yet obscure publication known as the quad-edge data structure^{199,200}. This data structure allows us to robustly handle the topology of the meshes we use in this work, which represent two-dimensional cross-sections of the hypocotyl of *Arabidopsis thaliana*. Based on the quad-edge data structure and topological terms, a more precise definition would be that our

meshes form an orientable, closed, two-dimensional manifold embedded in three-dimensional Euclidean space. This manifold has a genus of 0, meaning we are considering a spherical, topological surface with a defined "inside" and "outside" or "clockwise" and "anticlockwise" orientation, or in more colloquial words, a sphere without "holes".

The quad-edge structure is named due to employing directed edges with an origin and destination vertex as depicted in Figure 3.3A. The term "quad"-edge signifies that for each directed edge e , the mirrored, called "symmetric" edges and the two corresponding dual edges are stored. Consequently, for each directed edge e , we can directly access the references to its origin and destination vertices, its left and right faces (relative to the edge's direction), and the next outgoing edge from its origin. One might use different movement operations to transverse the entire mesh, starting on an edge e and progressing from there (Figure 3.3A,B). The powerful feature of this approach is that it not only trivializes navigating, for instance, around a polygon, but it also allows for the safe handling of topological changes like the merging of existing or the creation of new vertices and faces. These changes are, for example, required in the case of cell divisions, and the "making" or "killing" of edges to split or merge faces or vertices can be carried out with so-called Euler operators²⁰¹. These operators preserve the number of vertices V , edges E , and faces F of a polyhedron of genus 0 as described before, i.e., $V - E + F = 2$. Consequently, the topological validity of such operations is guaranteed within this data structure and a mesh always remains a manifold when using the Euler operators. From a technical perspective, this data structure represents a connected graph that can be implemented in C++ as a linked list of pointers²⁰¹.

In summary, while the biological model tissue considered here is a two-dimensional surface, geometrically represented in our model as a polygonal mesh, topologically, it still is a "spherical", flat surface. To close off this surface, a single boundary polygon (purple arrows in Figure 3.3B) is required that extends over all outermost edges. One might imagine, for instance, a hemispherical dome containing individual polygons on its hemispherical surface that is closed with a bottom "lid" underneath. To obtain a planar geometry, all z -values of all the polygons' vertices are set to zero, flattening the structure. At the same time, topologically, the mesh remains a sphere of zero volume.

In practice, when constructing meshes, we directly create flat geometries of the desired outline with the bottom boundary polygon and restrict the degrees of freedom of vertices to two dimensions.

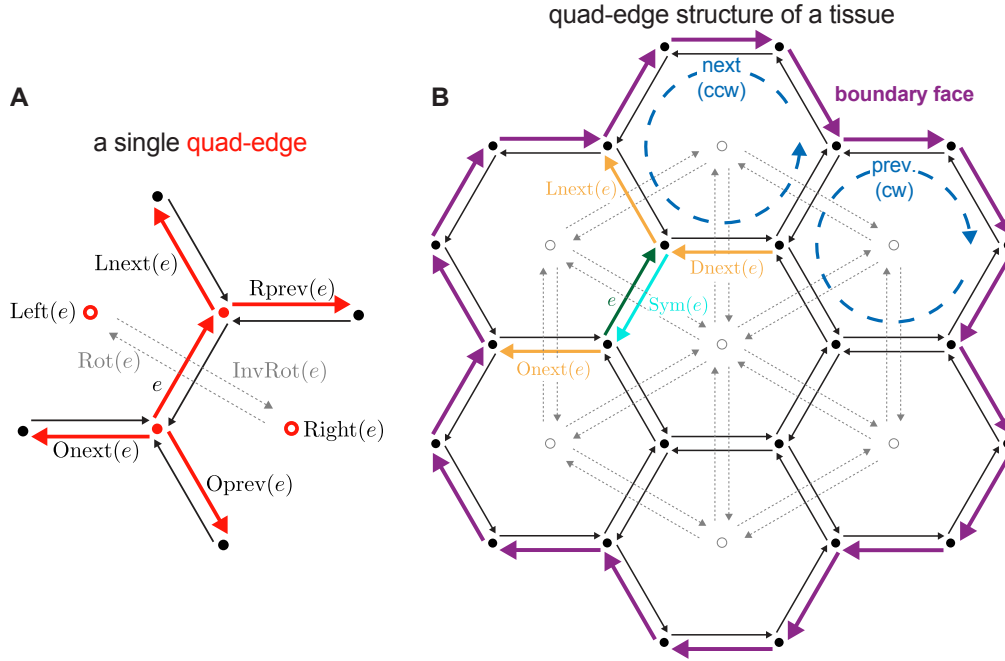


Figure 3.3: The quad-edge data-structure allows for a straightforward description of topology and geometry and navigation through a mesh. (A) Example of a single "quad-edge", storing for each directed edge e references to its origin and destination vertex (red dots), to its left and right faces (represented by red circles) and outgoing edges from the origin and destination vertices. By swapping faces and vertices, one might also obtain the dual, where, e.g., the destination of $Rot(e)$ is the same operation as considering the $Left(e)$. (B) An entire quad-edge structure is used to describe a mesh consisting of hexagons. From an edge e , one might traverse the mesh using "next" directions to traverse in counter-clockwise orientation or "prev" directions to traverse in clockwise orientation (dashed blue circles). One might use $Lnex(e)$ to iterate through the edges of the current polygon. With $Dnext(e)$ or $Onex(e)$, surrounding polygons can be accessed in a counter-clockwise orientation. Dashed straight lines show a part of the dual of the hexagonal mesh, which is a trigonal mesh. As the mesh topologically can be considered spherical, a boundary face (represented by purple arrows) is necessary to close off the mesh. Edges going to and from the boundary are not displayed.

3.2.2 Geometry

Since quad-edge provides us with an elegant way to treat any topological changes in the model, we only have to focus on the geometrical properties, i.e., the vertices and any physical quantities tied to them^c. Thus, it comes down to considerations of vertex positions $\mathbf{x}_i$ and their derived quantities. This is especially true since we intend to optimize the mechanical energy of the system, written in terms of its geometrical features. Note that, in general, many quantities

^cImportant note: The details of the geometrical considerations of the model used here, especially gradient computations, have been described in detail in the dissertation of João Ramos²⁰². The basic scaffold of the C++ implementation of the model was written by João Ramos as well, based on the earlier works of Jason Khadka⁶⁸ and Karen Alim. Furthermore, our modeling approach is a derivative of a very similar model introduced in⁶⁷. Consequently, we keep this section more general and discuss the aspects and features used during this work, focusing on those explicitly developed for this system.

are much easier to calculate and describe in two dimensions. As this model, in principle, also includes the capability for simulation of three-dimensional surfaces, those expressions become much more complex²⁰². Figure 3.4A,B depicts a few basic considerations and our labeling convention. Following the quad-edge data structure, a polygon α has vertices $i \in \mathcal{V}_\alpha$, labeled in a counterclockwise direction. For each cell, based on the vertex positions, we can directly compute quantities like the perimeter based on the Euclidean distances (Figure 3.4A). However, for most quantities we need to compute when establishing our mechanical growth model in Section 3.3, it is beneficial to subdivide each polygon into a set of triangles. In our choice of triangulation, each polygonal vertex connects to the centroid of the polygon (Figure 3.4B, grey lines). The significant benefit of this approach is to simplify computations of quantities that change when vertices are shifted. Since vertex shifts only affect the triangles that they are connected to, this approach potentially reduces some redundancy in our computations. More importantly, this approach is beneficial to compute gradients, for instance, area gradients and other time-dependent quantities that are required for efficient energy optimization in our mechanical model (cf. subsequent Section 3.3 and Reference²⁰²). For the resulting triangulated mesh, we use the following notation: for a given vertex i , the vertices orbiting around this vertex are denoted by j . Accordingly, we define the number of vertices around a face α by $\mathcal{N}_\alpha$ and the number of vertices of the triangulated mesh, orbiting around a vertex i as $\mathcal{T}_i$. To perform such a triangulation, as shown in Figure 3.4B in the first place, we need to determine the centroid geometrically.

Centroid computation

For two-dimensional polygons, the computation of centroids can be simplified by subdividing each polygon into triangles for which the areas and centroids are easy to calculate. By combining these triangles and weighting their centroids by their respective areas, the overall centroid of the polygon can be determined. To achieve this, we first compute the polygon's area using the shoelace formula, which allows for the calculation of the area of any arbitrary, non-intersecting, closed polygon^{203,204}. The underlying concept of this formula is illustrated in Figure 3.4C. The polygon is subdivided into triangles, each consisting of a fixed, arbitrary reference point, a vertex i , and a vertex $i + 1$. For each of these triangles, double its area is computed by the cross product of the points' location vectors. For the example in Figure 3.4C, the first four triangle areas computed this way (green faces) already include the entire polygon for which we aim to determine the area but also include an excess region outside of it (red faces, overlapping the green faces). Since we traverse the polygon in a counterclockwise direction, the last two triangle areas that are computed (red faces) have a switched sign because, for

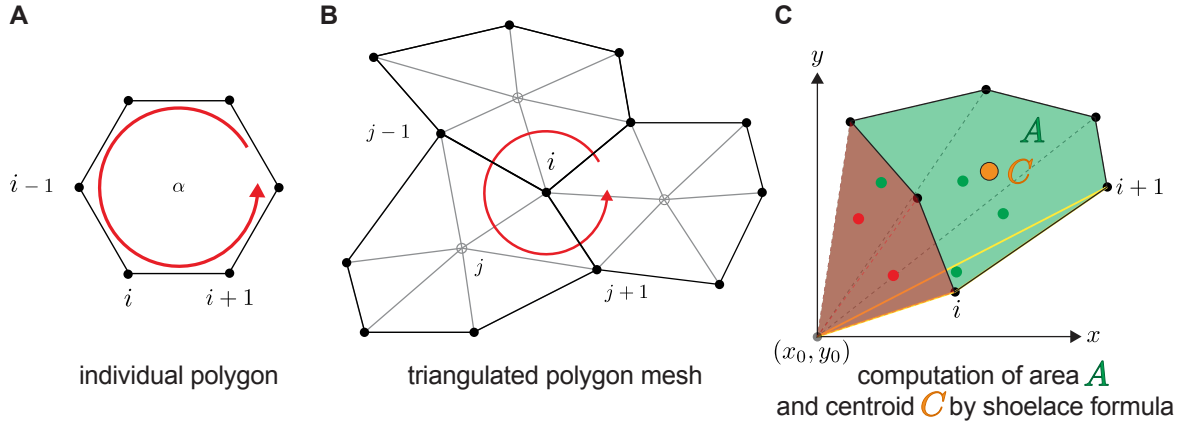


Figure 3.4: Geometry of polygonal meshes and shoelace formula. (A) For an individual polygon we denote with α the polygon itself and with i its vertices. (B) For many quantities, it is useful to triangulate polygons, using their centroid to connect to the polygon's vertices. For a given vertex i , we label the vertices orbiting around it with j . (C) To compute the centroid, we apply the shoelace formula, computing the area of each triangle, defined by a reference point (x_0, y_0) and two vertices $i \in \mathcal{V}_\alpha$ of the polygon. Faces in green represent triangles with a positive area contribution and faces in red with a negative contribution to account for excess areas. Note that the green faces overlap over the red ones towards the reference point (dashed triangle outlines) to cancel the excess areas precisely. The polygon's centroid (orange dots) is determined from the summation of all triangle centroids (green and red dots), weighted by their area.

them, the triangle between the reference point and the vertices is in a clockwise direction. Thus, this region is subtracted from the total area, yielding the correct area for the polygon. Writing down this procedure in terms of equations yields the shoelace formula^{203,204}:

$$A = \frac{1}{2} \sum_{i \in \mathcal{V}_\alpha} (x_i y_{i+1} - x_{i+1} y_i), \quad (3.8)$$

where $i \in \mathcal{V}_\alpha$ are the vertices of a polygon α . The summation is evaluated for all vertices, with the indices i and $i+1$ cyclically wrapping around the polygon such that the last vertex connects back to the very first. Each term of the sum corresponds then to the area of a triangle formed by two consecutive vertices and the origin, contributing to the total area of the polygon.

The centroid C that we aim now to calculate is an extension of the shoelace formula and can be computed by²⁰⁴:

$$C_x = \frac{1}{2A} \sum_{i \in \mathcal{V}_\alpha} \frac{(x_i + x_{i+1})}{3} (x_i y_{i+1} - x_{i+1} y_i) = \frac{1}{6A} \sum_{i \in \mathcal{V}_\alpha} (x_i + x_{i+1}) (x_i y_{i+1} - x_{i+1} y_i) \quad (3.9)$$

$$C_y = \frac{1}{2A} \sum_{i \in \mathcal{V}_\alpha} \frac{(y_i + y_{i+1})}{3} (x_i y_{i+1} - x_{i+1} y_i) = \frac{1}{6A} \sum_{i \in \mathcal{V}_\alpha} (y_i + y_{i+1}) (x_i y_{i+1} - x_{i+1} y_i) \quad (3.10)$$

where again, $i \in \mathcal{V}_\alpha$ are the vertices of a polygon α . The principle of the computation remains

the same compared to the shoelace formula. We again follow the path around the polygon and consider triangles that are defined by its vertices and some reference point. These terms are the same when compared with the shoelace formula. Additionally, we are including now the center of mass of each triangle (colored dots in Figure 3.4C), represented by the terms $(x_i + x_{i+1})/3$ and $(y_i + y_{i+1})/3$. Thus, the centroids are computed from the sum of all triangle centroids, weighted by the area of the respective triangle. In the following, we require the centroid of polygons to define local coordinates for each polygon.

Normal unit vector and projected coordinates

As mentioned in Section 3.2.1, topologically, we are considering a sphere using the quad-edge structure or a disc if considering two dimensions where the top of the disc represents the studied tissue and the bottom topologically closes the disc. Defining a normal unit vector $\mathbf{w}$ of a given polygon is useful to distinguish the bottom cell, i.e., the boundary cell, from other cells. We do so by summing and normalizing the cross products of consecutive vertices²⁰²:

$$\mathbf{w} = \frac{\mathbf{N}}{\|\mathbf{N}\|}, \quad \mathbf{N} = \sum_{i \in \mathcal{V}_\alpha} \mathbf{n}_i = \sum_{i \in \mathcal{V}_\alpha} (\mathbf{x}_i - \mathbf{X}) \times (\mathbf{x}_{i+1} - \mathbf{X}) = \sum_{i \in \mathcal{V}_\alpha} (\mathbf{x}_i \times \mathbf{x}_{i+1}). \quad (3.11)$$

with vertices $\mathcal{V}_\alpha$ of a polygon α , the vertex positions $\mathbf{x}_i = (x_i, y_i)$ and the polygon centroid $\mathbf{X} = (X, Y)$. In the two-dimensional case this allows us to distinguish the boundary, i.e., the bottom polygon "under" the disc (see boundary face in Figure 3.3B and Figure 3.5) by its normal unit vector $\mathbf{w}_{\text{boundary}} = -1$ which will be important for the mechanical model, described in Section 3.3. Furthermore, we define an intrinsic coordinate system for each polygon with the centroids as the origin and the normal unit vector being normal to the respective surface (cf. Figure 3.5), which will ultimately simplify computations, described in the following section. To obtain intrinsic coordinates, the centroids and normal vectors $\mathbf{w}$ allow us to derive a new basis of vectors $\mathbf{u}$ and $\mathbf{v}$ for each polygon by a Gram-Schmidt orthonormalization, using the unit vector $\vec{\mathbf{e}}_x$ ²⁰²:

$$\mathbf{u} = \frac{\mathbf{u}^*}{\|\mathbf{u}^*\|}, \quad \mathbf{u}^* = \vec{\mathbf{e}}_x - (\vec{\mathbf{e}}_x \cdot \mathbf{w})\mathbf{w}, \quad (3.12)$$

where $\mathbf{u}^*$ is the rejection of $\vec{\mathbf{e}}_x$ on $\mathbf{w}$. For the case of $\mathbf{w} = \vec{\mathbf{e}}_x$, $\vec{\mathbf{e}}_y$ is taken instead. Then, the last basis vector can be computed from the vector product

$$\mathbf{v} = \mathbf{w} \times \mathbf{u}. \quad (3.13)$$

With this approach, we can now project vertex positions to the local reference coordinate system, that is²⁰²:

$$\mathbf{x}_i^{\text{proj}} = (\mathbf{u} \cdot \mathbf{x}_i) \mathbf{u} + (\mathbf{v} \cdot \mathbf{x}_i) \mathbf{v} = (\mathbf{u} \cdot \mathbf{x}_i, \mathbf{v} \cdot \mathbf{x}_i) = (x_i^{\text{proj}}, y_i^{\text{proj}}). \quad (3.14)$$

With these coordinates, the projected centroid of the polygon and its projected area may be calculated by:

$$\mathbf{C}^{\text{proj}} = \frac{1}{6A^{\text{proj}}} \sum_{i \in \mathcal{V}'_\alpha} (x_i^{\text{proj}} y_{i+1}^{\text{proj}} - x_{i+1}^{\text{proj}} y_i^{\text{proj}}) (\mathbf{x}_i^{\text{proj}} + \mathbf{x}_{i+1}^{\text{proj}}), \quad (3.15)$$

$$A^{\text{proj}} = \frac{1}{2} \sum_{i \in \mathcal{V}'_\alpha} (x_i^{\text{proj}} y_{i+1}^{\text{proj}} - x_{i+1}^{\text{proj}} y_i^{\text{proj}}), \quad (3.16)$$

where the projected area A^{proj} is determined by the shoelace formula²⁰³. This leads us to the final expression for our projected coordinates:

$$\mathbf{x}'_i = \mathbf{x}_i^{\text{proj}} - \mathbf{C}^{\text{proj}} = (x'_i, y'_i), \quad (3.17)$$

to describe the position of individual vertices.

In our case of two-dimensional surfaces, the final result is an origin-shifted local coordinate

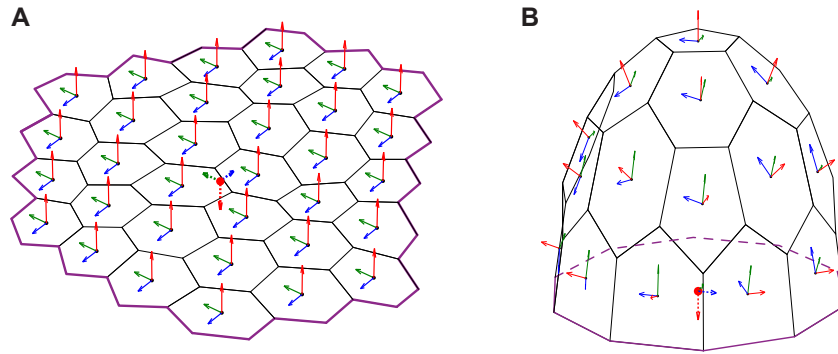


Figure 3.5: Projected coordinates in 2D and 3D surfaces. (A) Projected coordinates for a two-dimensional mesh. In this case, the intrinsic coordinate system does not need to be projected as the mesh is planar. Furthermore, in a two-dimensional mesh, the normal unit vector is always $\mathbf{w} = (0, 0, 1)$, except for the boundary (purple line with centroid indicated by red dot and dashed arrows) where it is $\mathbf{w} = (0, 0, -1)$. (B) Projected coordinates on a hemispherical mesh of polygons. For each polygon, the centroid defines an intrinsic coordinate system as a reference point for the projection. This also includes the boundary polygon at the bottom.

system as illustrated in Figure 3.5A, indicating the boundary polygon by its negative normal vector (dashed red arrows). Notably, if we were to consider three-dimensional meshes, this approach would also provide a means to reduce complexity to only two dimensions,

assuming individual polygons to be approximately planar²⁰². Consequently, this approach would produce a rotated, local coordinate system projected onto the three-dimensional surface with the polygonal centroids as a reference, as depicted in Figure 3.5B.

We further utilize these intrinsic coordinates in the following section to simplify computations that apply especially to the cell shape that we describe by the second moment of area. Using local coordinates and centering each cell in the origin of its unique coordinate system is both sufficient and computationally significantly easier for this purpose.

This concludes the basic description of geometry and topology, which we will use to describe the state of the mesh when conducting mechanical modeling of plant tissues.

3.3 Mechanical modeling in the cell-based vertex model

In the previous section, we introduced the tools to handle both topology and geometry, enabling us to formulate a mechanical model for modeling plant cell mechanics. As we aim to replicate real-world processes, combining math, computer science, physics, and plant biology, for simplicity, we will refer to polygons as "cells" and to polygonal meshes as "tissues" in the following. In short, as hinted in Section 3.1.2, beyond the sheer topological and geometrical descriptions, we set out to establish a mechanical model. Our approach of choice is a cell-based vertex model where we seek to optimize the mechanical state by re-positioning vertices to minimize a Hamiltonian that incorporates physical principles into our model. In the following, we will describe how we define the shape of cells and their connection to mechanical energy, as well as other contributions to the Hamiltonian and the relation of these quantities with classical continuum mechanics and constitutive relations. Furthermore, we describe the concepts and implementation of simulating growth and topological changes in the tissue. Based on the biological background of cortical microtubules and cellulose microfibrils, we also include mechanical feedback, cell division, and other topological transitions where no new cells are created.

3.3.1 Mechanical descriptions of plant cells

Minimizing tissue energy

In our cell-based vertex model, we want to capture plant cell mechanics and need a suitable mathematical description of the mechanical energy of the tissue. Here, the main contributors to the tissue energy are the elastic energy, i.e., mechanical energy from deformation, and the

turgor pressure. These and contributions from reasonable extensions to the model, such as an external potential, will be described in the following paragraphs. The state of the tissue as a whole, composed of individual cells and their form or shape, is the result of all these contributions. Each cell is defined by its vertex positions, which determine the individual contribution to the total tissue energy. Thus, in each simulation step, we seek the tissue's ground state by minimizing the tissue's total energy. More abstractly, we shift the vertex position vectors $\{\mathbf{x}_j\}$, defining the geometric outlines of the cells in our simulations, to find the energetically ideal configuration²⁰²:

$$\mathbf{x}_i \in \arg \min_{\mathbf{x}_j} \mathcal{H}(\{\mathbf{x}_j\}) \quad (3.18)$$

where $\mathbf{x}_i$ represent the vertex positions to minimize the Hamiltonian $\mathcal{H}$. Note that we follow a quasi-static approach, meaning that we take discrete steps from one stable configuration to the next without considering what is precisely happening in between. In more technical terms, we assume a succession of equilibrated states sufficiently approximates the system's dynamics. We argue this is reasonable because growth is slow, and the dynamics of cell divisions that we ultimately study cannot be captured in a mechanical model in its entirety with all intermediate biological stages (cf. Section 2.1.4). Consequently, we abstract the problem and only consider the states before and after a division process, where a new cell wall has fully formed, introducing additional vertices to the system. For our energy optimization, we consider these changes and carefully pick our parameters to avoid any sudden discontinuities of the tissue energy and configuration from sudden shifts of vertices. To determine how the vertex positions must be altered in each step to minimize tissue energy, we want to describe the different terms we incorporate into the Hamiltonian in the following paragraphs.

Shape matrices

As mentioned previously, the common ground for the contributions to the Hamiltonian is the description of the shape of the cells and their physical properties to bridge the gap between a pure geometrical mesh and an elastic tissue with biophysical features. Ultimately, we would like to connect the shape of cells to describe the tissue state using known quantities and constitutive relations from classical continuum mechanics. Beyond the pure description of polygons as a collection of connected vertices, we apply here the concept of shape matrices, for which we exploit the two-dimensional description of our local coordinates to simplify our calculations. We define two kinds of shape matrices: the current, deformed shape matrix M that represents the shape of cells at time t and the target shape matrix M_0 that describes the

shape a cell would take if it would reach its energy minimum. Both usually differ because, in a tissue, individual cells are constrained in their behavior by their neighbors. The difference between the current shape M and the target shape M_0 will ultimately govern our system's development over time. We apply here a very similar definition of the shape matrix to the one introduced by Uyttewaal et al.⁶⁷ and recently applied in other studies^{68,88,176}. We furthermore stick to using the local coordinates introduced in the previous section to define the shape matrix M for a single cell α with its components by:

$$M_{ij} = \iint_S x'_i x'_j dS, \quad (3.19)$$

where the integration is over the projected cell's region S , and the reference point is, by using the projected coordinates, the centroid of the cell. Here, we also dropped the cell index α for readability; however, all the following quantities that depend on the shape matrices or derived quantities are still considered for individual cells α . Furthermore, we represent vectors by $\mathbf{v} = v_i \mathbf{e}_i$, where $i = 1, 2$ corresponds to x and y and we assume Einstein summation convention. Thus, for each given cell α , the expression M represents a two-dimensional, symmetric matrix, i.e., contains three independent matrix entries. This matrix is, in essence, the second moment of area of the cell, computed in local coordinates with the cell's centroid as a reference point for the calculation. As hinted earlier, this procedure makes the computation much more manageable as no additional theorems have to be applied as would be required if not considering centroidal axes²⁰⁵. The second moment of area is a quantity well known in engineering for describing bending resistances and being closely linked to the inertia tensor^{206,207}. The benefit we gain from this is to obtain a means to describe the point distribution of a given cell. For the exact computation of the second moment of area, we can once more benefit from the shoelace formula²⁰³, which allows, as a special case of Green's theorem, to calculate the shape matrix for a cell α as:

$$M_{xx} = \frac{1}{12} \sum_{i \in \mathcal{V}'_\alpha} (x'_i y'_{i+1} - x'_{i+1} y'_i) (x_i'^2 + x'_i x'_{i+1} + x_{i+1}'^2), \quad (3.20)$$

$$M_{yy} = \frac{1}{12} \sum_{i \in \mathcal{V}'_\alpha} (x'_i y'_{i+1} - x'_{i+1} y'_i) (y_i'^2 + y'_i y'_{i+1} + y_{i+1}'^2), \quad (3.21)$$

$$M_{xy} = M_{yx} = \frac{1}{24} \sum_{i \in \mathcal{V}'_\alpha} (x'_i y'_{i+1} - x'_{i+1} y'_i) (x'_i y'_{i+1} + 2x'_i y'_i + 2x'_{i+1} y'_{i+1} + x'_{i+1} y'_i). \quad (3.22)$$

From these expressions, we may conclude that the second moment of area enables us to compare the shapes of cells regardless of the number of vertices and edges that define them

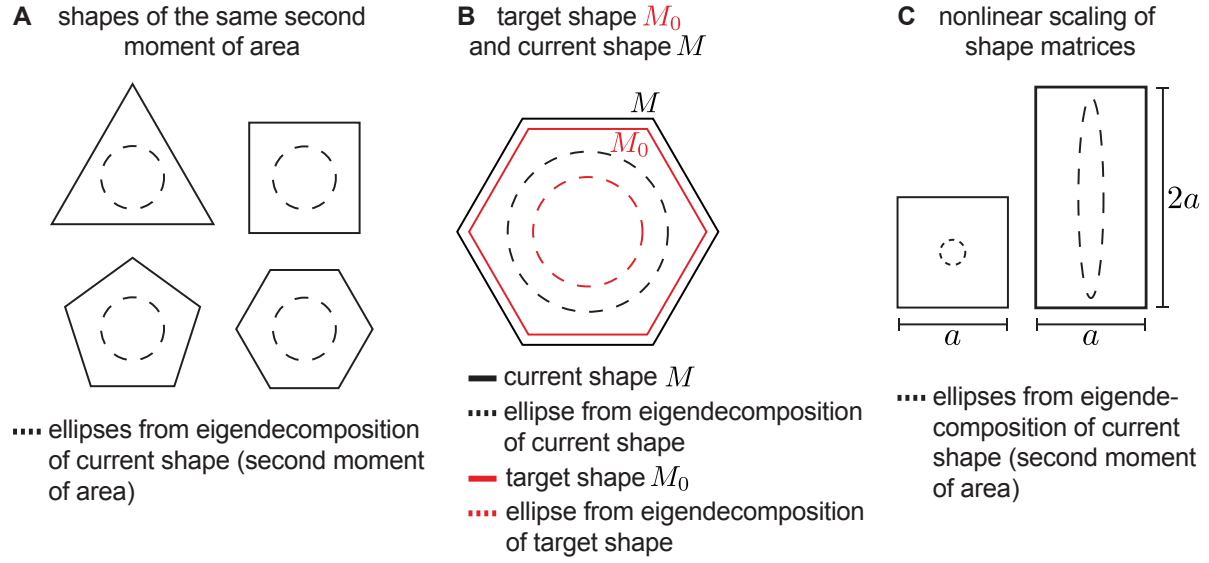


Figure 3.6: The second moment of area allows to define shape. (A) Different regular polygons and their second moment of area as defined in Eq. 3.19, represented by ellipses (dashed lines). Note that given the proper scaling, different shapes have the same second moment of area, i.e., shape matrix, regardless of their geometry. (B) Scaling of the second moment of the area under deformation of the considered polygon between its current shape M (black) and its target shape (red), i.e., the undeformed shape M_0 . Due to the highly nonlinear scaling, small deformations in cells lead to higher-order changes in the shape matrices M and M_0 . (C) Example of the highly nonlinear scaling of the shape matrix. For a uni-axial extension of a square, a simple deformation leads to a highly nonlinear change due to the scaling of the second moment of area of, in this case, order $\mathcal{O}(xy^3)$. Ellipses in panel (C) were scaled in size to fit inside the shapes.

because the shape matrices solely depend on the cell shapes and their (an)isotropy. As a result, this circumstance allows to avoid any bias from the number of edges toward our mechanical model. For a visual demonstration of this observation, we can also compare the shape of cells and their respective shape matrices as displayed in Figure 3.6A. For this comparison, we perform an eigendecomposition of the shape matrices, allowing us to construct ellipses representing the shape. For instance, a perfectly isotropic hexagon with equal sides and 120-degree angles would lead to a shape matrix of $M_{\text{hexagon}} = I$ if properly scaled in size. As a result, the ellipse which we can construct would be a circle of radius $r = 1$. Yet, other shapes, such as triangles, quadrangles, or pentagons, could have entirely the same second moment of area despite their very different geometry.

Note that compared to the standard definition of the second moment of area, we slightly deviate from it by switching the entries for xx and yy direction. We do this as we are not interested in bending resistance as engineers apply this quantity. Instead, we want to relate it to the shape of cells, therefore aligning the matrix entries with the axes geometrically. While this does not change anything regarding computing the tissue energy, as we will see in the

following, it provides us with means to define a strain in the system based on the difference of the current shape matrix M and the target shape matrix M_0 (cf. Figure 3.6B). We write the mismatch between the current shape and the target shape of a cell, also defined as their point distribution, as follows:

$$\Delta M = \frac{M - M_0}{\text{Tr}(M_0)}, \quad (3.23)$$

which can be considered a proxy of strain, as detailed in the following subsection.

Another property to consider regarding the shape matrices is their anisotropic nature. Besides the independence of the shape matrices on the number of vertices and edges, deformations lead to large relative changes of M and M_0 since the expressions for the shape matrix are of order $\mathcal{O}(xy^3, x^3y, x^2y^2)$ (Figure 3.6C). Thus, the differences in shape matrices, in relation to actual shapes have to be carefully approached when modeling deformations with shape matrices. While not explored in this work, this non-linearity requires additional considerations if dealing with highly anisotropic, i.e., elongated cells such as the ones one would find in longitudinal cross-sections of plant tissues like the stem or the roots, and can be circumvented to some extent by modeling of cell walls directly instead of cells as individual units of mechanical response as explored for example in^{57,202}. Our above approach is feasible for our purpose since we have relatively uniform cells and only very small deformations. In the upcoming section, we discuss the connection of the shape matrix formalism to classical continuum mechanics and infinitesimal strain theory.

Constitutive relations of strain and stress

The previously described shape matrix formalism provides several benefits, especially its intuitive description of shape and anisotropy when visualizing shape matrices and shape anisotropy by ellipses from eigenvectors and eigenvalues. Nevertheless, the entire shape matrix formalism is also quite phenomenological. Consequently, we wonder how to precisely relate the shape matrix to the quantities known from classical continuum mechanics, i.e., how to define strain, stress, and the relation of the two in the given context using the shape matrices. From classical continuum mechanics, considering the most simple case of a one-dimensional extension of $\Delta l = l - l_0$, strain is typically defined as follows¹⁶⁰:

$$\varepsilon = \frac{\Delta l}{l_0} = \frac{l - l_0}{l_0}. \quad (3.24)$$

In a more general case, the infinitesimal strain tensor ε_{ij} for small deformations is introduced to account for higher-dimensional considerations,

$$\varepsilon_{ij} = \frac{1}{2} \left(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right), \quad (3.25)$$

where $u_{i,j}$ are the displacement fields of a deformation, connected to the deformation gradient by $F = \nabla \mathbf{u} + I$ where I is the identity matrix. Thus, it is defined via the displacement $\mathbf{u}$, i.e., the spatial difference of a material point between its position in its rest configuration and the current position. By its definition, the strain tensor is symmetric. From this tensor ε , describing the deformation of a solid body, the components of the also symmetric stress tensor σ , describing a measure of force over an area, can be computed. Hooke's law relates both tensors, defining the constitutive relation between strain and stress as follows¹⁶⁰:

$$\sigma_{ij} = C_{ijkl} \varepsilon_{kl}, \quad (3.26)$$

where C_{ijkl} represents the stiffness tensor containing the material properties of the solid body under consideration. In the most simple case of a linearly elastic solid that we are considering in this work^d, this boils down to only two independent terms remaining in the stiffness tensor: the first and second Lamé parameters λ and μ . Thus, the constitutive relation between strain and stress is reduced simply to

$$\sigma_{ij} = C_{ijkl} \varepsilon_{kl} = 2\mu \varepsilon_{ij} + \lambda \varepsilon_{kk} \delta_{ij}. \quad (3.27)$$

In addition to strains and stresses, we furthermore may write down the elastic energy or strain energy per unit volume of the deformation of a solid, which is¹⁶⁰:

$$u = \frac{1}{2} C_{ijkl} \varepsilon_{ij} \varepsilon_{kl}, \quad U = \int_V u dV, \quad (3.28)$$

or for the case of the linear elastic solid and expression via the Lamé parameters accordingly

$$U = \frac{1}{2} (2\mu \varepsilon_{ij} \varepsilon_{ij} + \lambda \varepsilon_{kk}^2), \quad (3.29)$$

^dIn brief, we take this approach because of the unavailability of information about any inhomogeneities of the plant material and because we are only considering tiny deformations.

or using the matrix notation

$$U = \frac{1}{2} (2\mu \text{Tr}(\varepsilon^2) + \lambda [\text{Tr}(\varepsilon)]^2). \quad (3.30)$$

The above expressions provide the basics of classical continuum mechanics of linear elastic solids. In principle, we could use these concepts to define the strain by using the deformation of a cell, which, in this sense, is considered an individual solid body. Such we could achieve by taking the mismatch between M and M_0 to define the strain, i.e., approximating a relation similar to $\varepsilon \approx \Delta M = (M - M_0) / \text{Tr}(M_0)$ for reflecting a deformation. Nevertheless, despite the connection of the target shape and current shape to the deformed and undeformed configuration that is the concept behind the strain, we have to properly connect the shape matrices to infinitesimal strain theory. Only then can we compare our model and its parameters and results with experimental research and other modeling approaches.

We also note that using the shape matrices M and M_0 , we cannot define a unique rotation and strain simultaneously; a simple rotation could also be achieved by specific stretching and contracting to achieve the same numerical result. Therefore, we already have to assume that we are considering small strains and that the mismatch between M and M_0 does not involve simple rotations.

Still, we would like to find a more precise connection between the strain and the shape matrix formalism. Such a relation was already derived in its full extent and length by João Ramos in²⁰². Therefore, we will primarily explain the main idea behind this derivation and point out the results here.

We start by reconsidering the concept of strain. In essence, strain is a deformation of a solid body, i.e., if remaining in the linear elastic regime, we can approximately describe a linear strain ε from its Taylor expansion as a linear transformation $\Lambda_{ij} = \delta_{ij} + \varepsilon_{ij}$ to transform a variable $x'_i = \Lambda_{ij}x_j$. Thus, the components of the second moment of area can be transformed according to the definition in Eqn. 3.19 by²⁰²:

$$M'_{ij} = \iint_{S'} x'_i x'_j dx'_i dx'_j = \iint_S \Lambda_{ik} x_k \Lambda_{jl} x_l \det(\Lambda) dx_k dx_l = \det(\Lambda) \Lambda_{ik} M_{kl} \Lambda_{jl}. \quad (3.31)$$

The determinant has to be included here as it is the Jacobian of the variable transformation. As a result, we can describe the current shape matrix M in relation to the target shape M_0 by

$$M_{ij} = \det(\Lambda) \Lambda_{ik} M_{kl,0} \Lambda_{jl}. \quad (3.32)$$

Beyond this section, we will also exploit this idea of linear transformation, using strain, for further considerations, for instance, when describing topological transitions. Assuming small deformations and thus staying inside the linear elastic regime, we can remove the nonlinear terms. Noting that $\det(I + \varepsilon) \approx 1 + \text{Tr}(\varepsilon)$, we obtain:

$$M_{ij} \approx \varepsilon_{mm} M_{0ij} + M_{0ij} + \varepsilon_{ik} M_{0kj} + \varepsilon_{jl} M_{0il}. \quad (3.33)$$

Solving for the strain ε results in the following expression, in matrix representation²⁰²:

$$\varepsilon = \frac{M - M_0}{\text{Tr}(M_0)} - \frac{1}{2} \frac{M_0}{\text{Tr}(M_0)} \frac{\text{Tr}(M_0) \text{Tr}(M - M_0) - M_0 : (M - M_0)}{\det(M_0)} - \frac{1}{2} I \left(\frac{\text{Tr}(M - M_0)}{\text{Tr}(M_0)} \right) + \frac{3}{8} I \left(\frac{\text{Tr}(M_0) \text{Tr}(M - M_0) - M_0 : (M - M_0)}{\det(M_0)} \right), \quad (3.34)$$

where the colon $:$ denotes the Frobenius product between the matrices, i.e., the component-wise inner product to return a scalar as $A : B = \sum_{i=1}^n \sum_{j=1}^m A_{ij} B_{ij}$.

This expression for the strain finally allows us to connect strain to the shape matrix formalism. Furthermore, we may use this connection to compute all derived quantities such as stress σ and, with it, the energy density and the elastic energy of the tissue by summation over the elastic energy of each cell. Thus, connecting strain and shape matrices allows us to apply classical continuum mechanics to our mechanical modeling.

Hamiltonian: Elastic energy and turgor pressure

In the previous section, we determined the connection between shape matrices and strain, enabling us to write down the energy contributions to our Hamiltonian in terms of strain. The energy is mainly composed of two terms: elastic energy from deformations and turgor pressure, which is an isotropic force. The elastic energy is the term introduced in Eqn. 3.30, integrated over the volume of a cell, i.e., the energy contribution from an individual cell is:

$$\mathcal{U}^{\text{elastic}} = \frac{1}{2} A_0 h (2\mu \text{Tr}(\varepsilon^2) + \lambda [\text{Tr}(\varepsilon)]^2), \quad (3.35)$$

where we use the strain as defined in Eqn. 5.2, A_0 denotes the area of a cell in its undeformed state and h the height of a cell. As we are considering a two-dimensional system, we do not include any volume effects and, therefore, set the height to $h = 1$. For practical reasons, we can also rewrite the elastic energy to allow modeling based on typically measured real-world parameters. For this purpose, we may replace the Lamé parameters with Young's modulus E_0

and Poisson's ratio ν using the relations¹⁶⁰:

$$\mu = \frac{E_0}{2(1 + \nu)}, \quad \lambda = \frac{E_0\nu}{(1 + \nu)(1 - 2\nu)}. \quad (3.36)$$

Typically, it is challenging to find precise measurements of physical parameters in plant tissue, especially because those may substantially change by orders of magnitude, depending on the tissue studied. For this work, we apply parameters from other modeling work and different measurements to estimate our mechanical parameters to $E_0 \approx 300$ MPa⁸⁵ and $\nu \approx 0.3$ ^{46,66}.

The second contribution to the Hamiltonian, described from a more biological perspective in Section 2.2.1, is the turgor pressure P , i.e., the internal pressure of an individual cell. To derive an expression for the energy contribution, we can consider the first law of thermodynamics, more precisely, the work done by this pressure. For this, a simple integration over the volume of a cell yields the energy contribution

$$\mathcal{U}^{\text{turgor}} = -PA_0h, \quad (3.37)$$

where again, due to the two-dimensional nature of this modeling, we set $h = 1$. Note that the turgor pressure P can also be cell-specific when modeling cell ablation. Considering a three-dimensional surface, adding another term for any internal pressure exerted on the simulated surface cells from internal cells and tissues would be reasonable. Based on existing modelling and measurements^{20,46}, we estimate the turgor pressure to be approximately $P \approx 0.5$ MPa.

Beyond the elastic energy and the contribution from the turgor pressure, we can make this model more comprising by adding further terms, such as an external potential as applied in simulations of spatially confined tissues in Section 5.5.1. We can include the contribution of any arbitrary, additional, external potential to the Hamiltonian as:

$$\mathcal{U}^{\text{pot}} = A_0U^{\text{pot}}(\mathbf{X}), \quad (3.38)$$

where the potential function U_{pot} is a function of the centroid $\mathbf{X} = (X, Y, Z)$ of a cell and, therefore, implicitly, a function of the cells' vertex positions x_i . This function must fulfill at least the requirement to be continuously differentiable to allow computing gradients for energy optimization. Additional requirements depend on the context. Here, we aim for a potential well to add the possibility of confined tissue growth. For this purpose, we chose a

one-dimensional logistic function in x-direction:

$$U^{\text{pot}}(X) = \frac{a}{(1 + \exp((X - \sigma_1)b))} + \frac{a}{1 + \exp(-(X - \sigma_2)b))}, \quad (3.39)$$

where the parameters σ_1 , σ_2 and b control the effective bounds and steepness of the potential and the amplitude a its depth. These parameters must be adapted to the tissue considered to achieve the desired effect.

In total, this leaves us with the effective expression for the Hamiltonian of our two-dimensional simulations of a tissue composed of n cells α of:

$$\mathcal{H} = \sum_{\alpha=1}^n \mathcal{U}_{\alpha}^{\text{elastic}} + \mathcal{U}_{\alpha}^{\text{turgor}} + \mathcal{U}_{\alpha}^{\text{pot}}, \quad (3.40)$$

$$= \sum_{\alpha=1}^n \frac{1}{2} A_{0,\alpha} (2\mu \text{Tr}(\varepsilon^2) + \lambda [\text{Tr}(\varepsilon)]^2) - \sum_{\alpha=1}^n P_{\alpha} A_{0,\alpha} + \sum_{\alpha=1}^n A_{0,\alpha} U_{\alpha}^{\text{pot}}(\mathbf{X}_{\alpha}), \quad (3.41)$$

or alternatively, in terms of Poisson's ratio and Young's modulus:

$$\mathcal{H} = \sum_{\alpha=1}^n \frac{1}{2} A_{0,\alpha} \left(\frac{E_0}{(1 + \nu)} \text{Tr}(\varepsilon^2) + \frac{E_0 \nu}{(1 + \nu)(1 - 2\nu)} [\text{Tr}(\varepsilon)]^2 \right) - \sum_{\alpha=1}^n P_{\alpha} A_{0,\alpha} + \sum_{\alpha=1}^n A_{0,\alpha} U_{\alpha}^{\text{pot}}(\mathbf{X}_{\alpha}), \quad (3.42)$$

where the first two terms always apply, and the third term only applies if an external potential is applied to alter the tissue dynamics.

In essence, the interpretation of these terms can be considered the following way: the elastic energy is the contribution that depends on the shape and deformation, i.e., strain acting on the cells. This energy may propagate from one cell to neighboring cells and those further away. Optimizing here tries to create an equilibrated state with a force balance, i.e., vanishing net force on each vertex. The potential term, if applied, can be considered an additional constraint on this force balance. The cell centroid, and by extension, the individual vertex positions, matter and influence the ideal configuration at every simulation step. Nevertheless, the effective parameter that drives growth is the turgor pressure. This applies to both the biology and mechanical simulation perspective, as turgor is necessary to generate the strains needed for growth. In turn, the elastic energy resists this expansion and deformation and governs the resulting cell shape upon such an expansion or as a result of external mechanical stimuli.

On a final note, beyond the Hamiltonian, one vital component to ensure efficient local

optimization of the energy is to provide gradients for the dynamic quantities. The significance of these gradients lies in their role as inputs for the optimization process to probe the energy landscape. A detailed derivation of these is elaborated in the dissertation of João Ramos²⁰².

3.3.2 Cellular growth dynamics

Cell growth function

The still missing element at this point is a growth model for how cells evolve within the established framework. Since the elastic energy depends on the strain ε , and the strain, in turn, depends on the mismatch between the shape matrices M and M_0 , we need to find a way to evolve M_0 over time. Without a growth model to evolve M_0 , we would otherwise converge toward a steady state without any further expansion in the absence of any other forces. The approach we chose here is similar to the Lockhart description of plant cell growth¹⁵⁵, and that has been applied in previous works by^{67,68,176}. In brief, as described in Section 2.2.2, plant cell growth and cell wall yielding is a continuous process but can be broken down into several steps. First, the turgor pressure increases, which puts additional stress on the cell walls. Then, at some threshold, the cell walls yield to this stress to increase the cell size and relax the stress on the walls, temporarily decreasing the turgor pressure, which is then quickly re-established. This process happens repeatedly and was described in an early theoretical model by Lockhart¹⁵⁵. From a physical perspective, this behavior corresponds to a Bingham plastic, which acts as a solid for low applied (shear) stress and a viscous fluid for larger stresses²⁰⁸. In the spirit of the Bingham plastic and considering that due to the rigid cell walls, plant cells typically do not shrink^e, we are looking for a description of the growth based on strain, where cells monotonously grow. We achieve this by evolving the target shape matrix M_0 , inspired from^{67,68,176} as:

$$\frac{1}{\text{Tr}(M_0)} \frac{dM_0}{dt} = \kappa \left(\frac{M - M_0}{\text{Tr}(M_0)} - \dots \right) = \kappa \varepsilon, \quad (3.43)$$

using the expression for the strain given in Eqn. 5.2. We normalize M_0 by its trace as the strain is also a function of M and M_0 , normalized by the trace of M_0 . Consequently, we may avoid an undesired cell size dependency by this normalization. In principle, the scalar growth factor κ can be any chosen modulation to the strain-based growth. Our choice for this

^eThis statement assumes turgid cells and no sources of substantial mechanical conflicts, i.e., no dehydration, cell wall degradation, softening or high salt concentration that might substantially affect the cell volume and tissue structure.

modulation we describe in Section 4.2. This factor also includes a baseline growth rate drawn from a Gaussian distribution $\mathcal{N}(\mu_g, \sigma_g^2)$ to ensure stochasticity of growth on a cellular scale to account for biological variation. To avoid shrinkage of cells, we furthermore perform an eigendecomposition of the entire term on the right-hand side of Eqn. 5.4, where we enforce the eigenvalues to be zero if negative, i.e. ⁶⁸:

$$(\kappa\varepsilon)_+ = V\Omega V^{-1}, \quad \text{with} \quad \Omega_i = \max(\omega_i, 0)I, \quad (3.44)$$

where V is the 2×2 matrix of eigenvectors and Ω the matrix of eigenvalues ω_i . Thus, the governing growth equation for evolving M_0 becomes:

$$\frac{1}{\text{Tr}(M_0)} \frac{dM_0}{dt} = (\kappa\varepsilon)_+, \quad (3.45)$$

ensuring the entire term on the right-hand side is positive semi-definite to provide a contribution to continuous growth. This procedure of utilizing the eigendecomposition of our growth equation to ensure positive-semidefinite expressions, we apply throughout this work, independently of the growth factor κ or further additions to the right-hand side of Eqn. 3.45.

Mechanical feedback

To construct a model that is as realistic as possible, we include the biological anisotropy of cell walls in the model by adding mechanical feedback. As described in Section 2.2.3, cortical microtubules affect the direction of cellular growth by guiding the deposition of cellulose microfibrils along the cortical microtubule orientation, ultimately changing the cell mechanics and leading to anisotropic growth in the direction perpendicular to stress. Considering earlier works^{67,68,89}, in principle, several options would come to mind for achieving this in our simulation. The main idea we want to capture is changing the growth direction, i.e., the shape of cells in response to the mechanical stress they experience, following the concept behind growth modulation by cortical microtubules. Thus, the quantity of interest to which we aim to couple cellular growth to, is the deviatoric stress tensor in 2D,

$$D = \sigma - \frac{\text{Tr}(\sigma)}{2}, \quad \text{or} \quad (3.46)$$

$$D_{ij} = \sigma_{ij} - \frac{1}{2}\delta_{ij}\sigma_{kk}, \quad (3.47)$$

where the second term in these equations is the hydro-static part of the stress tensor, related to the volume change of a solid body. In earlier works this tensor has also been chosen to

describe the modulation of growth either in combination with the target shape M_0 only^{67,89} or the mismatch of $M - M_0$ for a representation of Lockhart-like growth⁶⁸ to mimic the influence of strain and, therefore, stress on growth. Using the shape matrices will also be our approach to consider the influence of strain and stress. We exploit that we may write strain ε as a function of M and M_0 . Consequently, we can rewrite the deviatoric stress in terms of strain, using Hooke's law as follows:

$$D_{ij} = 2\mu\varepsilon_{ij} + \lambda\delta_{ij}\varepsilon_{kk} - \frac{1}{2}\delta_{ij}(2\mu\varepsilon_{kk} + 2\lambda\varepsilon_{kk}) = 2\mu\left[\varepsilon_{ij} - \frac{\delta_{ij}}{2}\varepsilon_{kk}\right], \quad (3.48)$$

where we expressed the deviatoric stress with Lamé's parameters instead of Poisson's ratio and Young's modulus for better readability. From here, a few options present themselves to modulate the growth function in Eqn. 3.45, adapting how M_0 evolves. The first option is to add a term to the growth equation, coupling the deviatoric stress directly to strain similarly to Khadka et al.⁶⁸. Such a coupling introduces a feedback term proportional to $D\varepsilon$ with some feedback parameter η to adjust its strength. Plugging in the terms for this approach and retaining the constraint of the entire growth function being positive semi-definite (Eqn. 3.44), we obtain

$$\frac{1}{\text{Tr}(M_0)} \frac{dM_{0,ik}}{dt} = \kappa\varepsilon_{ik} - \eta D_{ij}\varepsilon_{jk} = \kappa\varepsilon_{ik} - 2\eta\mu\left[\varepsilon_{ij}\varepsilon_{jk} - \frac{\varepsilon_{ik}}{2}\varepsilon_{ll}\right].$$

This captures stress-strain-related mechanical feedback with the deviatoric stress, effectively acting as a modulation of the strain without feedback. However, an intuitive understanding of coupling strain to deviatoric stress proves challenging, and we observe that nonlinear terms arise from this expression if we rewrite the deviatoric stress in terms of strain.

Suppose a uni-axial strain without shear, i.e., ε in xx direction with the resulting strain in yy -direction, assuming a Poisson ratio^f of $\nu > 0$. Then, we obtain the resulting strains $\varepsilon_{xx} = \varepsilon$ and $\varepsilon_{yy} = -\nu\varepsilon$. Plugging these into our growth equation yields:

$$\begin{aligned} \frac{1}{\text{Tr}(M_0)} \frac{dM_{0,xx}}{dt} &= \kappa\varepsilon - 2\eta\mu\varepsilon^2, \\ \frac{1}{\text{Tr}(M_0)} \frac{dM_{0,yy}}{dt} &= -\kappa\nu\varepsilon - 2\eta\mu\nu^2\varepsilon^2, \end{aligned}$$

meaning the expression for the target shape change dM_0/dt becomes parabolic. Thus, the shape matrix change in the yy direction for a non-zero strain would be strictly negative.

^fRealistic values of Poisson ratios for plant cells are around $\nu \approx 0.3$ ⁴⁶.

Consequently, as we enforce the expression to be positive semi-definite, the term would always be zero. In the xx direction, the term would also be zero if the strain exceeds a threshold of $\varepsilon = \kappa/(2\eta\mu)$. Thus, the effect of the feedback depends on the specific choice of the parameters κ and η . With this approach, we might run into the risk of not only stopping growth perpendicular to the direction of strain and stress but also leading to the stagnation of growth if strains become too large in the tissue. Hence, this would contradict our goal of including a microtubule-induced mechanical response in the model because the aim is to enhance growth in the direction perpendicular to stress. While this scenario of stagnating growth is only one specific case, which we expect to be only marginal, we aim to avoid it entirely.

To prevent such potential issues and nonlinear terms, we decide to couple the evolution of the target shape M_0 immediately to the deviatoric stress tensor D as a means of direct mechanical feedback. Our growth function, including the mechanical feedback term, is then represented by:

$$\frac{1}{\text{Tr}(M_0)} \frac{dM_{0,ik}}{dt} = \kappa \varepsilon_{ik} - \eta D_{ij}, \quad (3.49)$$

$$= \kappa \varepsilon_{ik} - 2\mu\eta \left[\varepsilon_{ik} - \frac{\delta_{ij}}{2} \varepsilon_{kk} \right], \quad (3.50)$$

leading in the previously considered marginal case of uni-axial strain to:

$$\frac{1}{\text{Tr}(M_0)} \frac{dM_0^{xx}}{dt} = \kappa \varepsilon - 2\mu\eta \varepsilon, \quad (3.51)$$

$$\frac{1}{\text{Tr}(M_0)} \frac{dM_0^{yy}}{dt} = -\kappa \nu \varepsilon + 2\mu\eta \nu \varepsilon, \quad (3.52)$$

thus contributing to reducing growth in the direction of mechanical stress and promoting uniform growth. Notably, in this implementation of feedback, growth may, in principle, still stagnate in the considered marginal case. However, on the one hand, this issue is confined to the yy direction, and on the other hand, it can potentially be resolved by adjusting the mechanical feedback. Moreover, our approach allows for precise feedback control by carefully tuning its strength η in relation to the other parameters.

3.3.3 Topological transitions

Topological transitions are an ever-occurring process in tissue morphogenesis and an important part of any mechanical model of morphogenesis and proliferation. In general, one can

distinguish between cell divisions and topological transitions that lead to a change in the neighborhood of existing cells without producing new cells, as shown in Figure 3.7. This work focuses primarily on cell divisions (Figure 3.7A) for their greater relevance in plant tissues. T1 transitions (Figure 3.3.3B), where cells change their neighborhood, are specifically a phenomenon of cell migration and of little relevance in plant tissues^{114,209}. In contrast, despite plant cells not migrating in the way that animal cells do, some processes demonstrate the existence of topological transitions in rigid structures beyond cell division. One example of such transitions is intrusive growth in *Arabidopsis* inflorescence stems¹¹⁴. Intrusive growth refers to individual cells expanding into existing tissue, progressively separating cells previously connected by adjacent cell walls. Conversely, they also form new connections with surrounding cells as they integrate into the structure (cf. Fig. 3.7C).

Cell divisions

In this work, we will consider the three types of cell divisions displayed in Figure 3.8, which are based on different division rules: maximum stress, short-axis, and random division. In each case, the division splits the cell into two parts, and the newly formed division wall intersects with the centroid of the mother cell.

The first step is always to determine the division plane. We compute the division plane

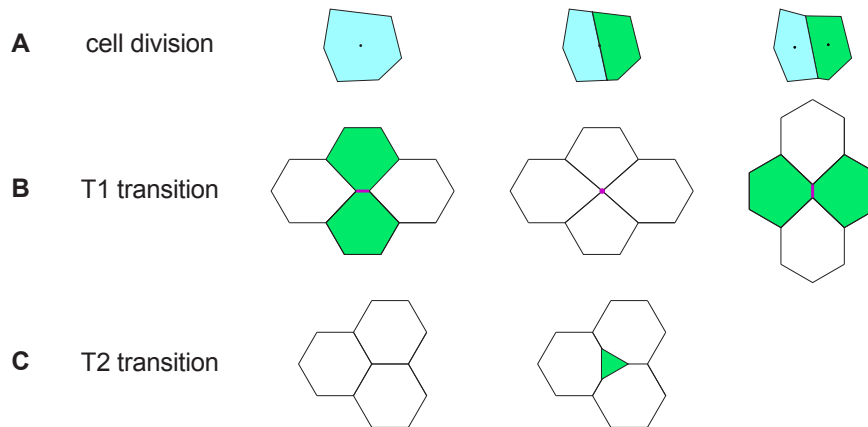


Figure 3.7: Topological phase transitions introduce new cells or change the neighborhood of existing ones. **(A)** In the case of a dividing cell, the division plane is chosen according to a chosen rule, dividing the cell through its centroid. In the process, new vertices, i.e., degrees of freedom, may be created (visualized by small indentations at the ends of the new cell wall in the image on the right). **(B)** Cells undergoing a T1 transition, as typically observed for migrating animal cells, have one cell wall shrinking (purple line) with the subsequent generation of a new one, changing their neighborhood (filled polygons) in the process. **(C)** T2 transition in a tissue where, from a two-dimensional perspective, a cell (filled polygon) is placed in between existing ones, for instance, by intrusive growth.

orientation for maximum stress division by considering a mother cell's stress tensor σ . To determine the direction of maximum stress, we perform an eigendecomposition to determine eigenvectors and eigenvalues. The largest eigenvalue then corresponds to the eigenvector, representing the direction of maximum stress. This direction and the centroid of the mother cell uniquely define a plane, which we will choose as the plane of cell division (Figure 3.8A,B). The difference between the largest and smallest eigenvalue also allows us to define stress anisotropy as a measure of the stress distribution within an individual cell.

Similarly, we proceed for short-axis divisions (Figure 3.8A,C), the only difference being that instead of the stress tensor, we utilize the current shape matrix M for which again we perform an eigendecomposition to find the smallest eigenvalue and its corresponding eigenvector. Picking the largest eigenvalue and corresponding eigenvector would allow us to compute the cell's longest axis. This procedure can be generalized for any quantity represented in a 2×2 matrix in a Cartesian coordinate system and is essentially the same as in earlier works^{67,89,176}. Finally, for random divisions, we select a random direction (Figure 3.8D). After determining the orientation of the new division plane, we then compute the intersection between this plane and the edges of the cell to divide to determine where the new vertices to define the new wall should be placed.

Besides the geometrical considerations, we must ensure that the mother cell's previous mechanical state before division adequately reflects the daughter cells after division. In this work, we will assume the mechanical and material properties, i.e., Young's modulus, Poisson ratio, and turgor pressure, remain unchanged except when explicitly stated otherwise. After cell division, the rest shape $M_0^{d_i}$ of the resulting daughter cells and their mechanical state needs to

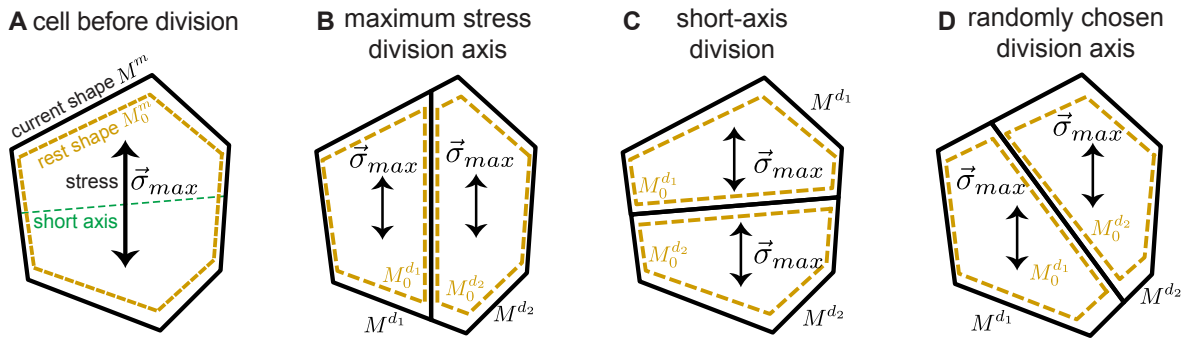


Figure 3.8: Cell division with three different division rules **A)** Mother cell with current shape M (black polygon) and target shape M_0 (yellow polygon with dashed outline) with the indication of the directions of maximum stress (black arrow) and short axis (dashed line). **(B-D)** Cell division in the direction of maximum stress (B), in the direction of the short axis (C), and random division (D). Figure adapted from¹³⁰.

be determined. Since a mother cell has a current shape M^m and a target shape M_0^m from where we previously (Eqn. 5.2 and following) derived the current strain and stress that the cell is subject to, we would like to have daughter cells M^{d_i} inherit the mechanical state of the mother cell. Recapitulating the shape matrix to be the second moment of area (Eqn. 3.19), we need to be aware of its nonlinear terms from which we computed it. Because of this nonlinearity, a simple approach to divide the current shape and target shape of the mother cell, e.g., like $M^m \neq M^{d_1} + M^{d_2}$ is not feasible and would not reflect the actual change in geometry and shape. From our considerations for the transformation of the second moment of area (Eqn. 3.32), we concluded that the connection between the current shape M and the target shape M_0 can be written in terms of a linear transformation $\Lambda_{ij} = \delta_{ij} + \varepsilon_{ij}$. This connection holds for any cell; therefore, it also applies to daughter cells. From Eqn. 3.32, we already know how the shape matrices transform. Thus, we only need to invert the transformation and compute the inverse of the linear transform Λ . Because it is only a 2×2 matrix, we may compute the inverse directly as follows:

$$\Lambda^{-1} = \frac{1}{\Lambda_{xx}\Lambda_{yy} - \Lambda_{xy}^2} \begin{pmatrix} \Lambda_{yy} & -\Lambda_{xy} \\ -\Lambda_{xy} & \Lambda_{xx} \end{pmatrix}. \quad (3.53)$$

Then, the overall transformation to find M_0 , based on knowing the strain that is embedded within Λ^{-1} and is acting on a cell, can be computed as

$$M_0^m = \Lambda^{-1} M^m (\Lambda^{-1})^T \det(\Lambda^{-1}), \quad (3.54)$$

$$M_0^{d_i} = \Lambda^{-1} M^{d_i} (\Lambda^{-1})^T \det(\Lambda^{-1}), \quad (3.55)$$

for both the mother and daughter cells. Besides the simplicity of this approach, a useful and important aspect here is that by applying the strain of the mother to both daughter cells, the mechanical energy does not change upon cell division as the total area and strain on this region do not change, meaning there will not be any discontinuities in the total energy. Still, performing cell division means introducing additional degrees of freedom via adding vertices along the path of the mother cell unless new vertex positions coincide with existing ones. Consequently, new states in the tissue energy become available.

Here, we assumed that daughter cells inherit strain and the mechanical properties of the mother cell for consistency with our strain-based growth model. Alternatively, one could also consider the case where stress is conserved instead of strain, and the mechanical properties of daughter cells differ from the mother cell. Then, the strain would have to be determined by trying to solve the linear set of equations to connect both $\sigma_{ij}^m = C_{ijkl}^{d_i} \varepsilon_{kl}^{d_i}$. Subsequently, $M_0^{d_i}$

could again be determined from the transformation in Eqn. 3.55.

Other topological transitions

Beyond cell divisions, one could imagine other topological transitions, especially if we were to adapt the model for applications such as epithelial tissue or animal cells in general. Even though we never applied them for the plant-tissue simulations in this work, we also included T1 transitions (cf. Figure 3.7B) as a proof of principle to the model. The approach is very similar to the one applied for cell division but involves four cells: the ones to the left and right of a given wall and the ones adjacent to each end (cf. Figure 3.7B). For each cell, to avoid discontinuities in the total energy, we may infer the target shapes M_0 of each cell via the transformation (Eqn. 3.54) described in the previous subsection. In these cases, the cell area changes in addition to the target shape matrices, leading to a slight offset in the total energy as all terms depend on the undeformed area A_0 .

Because of the absence of migration in plant cells, such transitions do not occur, and we did not include them into simulations for this project. In some cases, however, it may be necessary to remove individual cell walls. Even though we enforce the growth equations to be positive semi-definite (Eqns. 3.44, 3.49), individual walls may still shrink during a simulation. Such shrinking is an effect of the interaction between cells as a part of the emerging morphological changes, especially in cases where growth is constrained in some way, like an external potential. In general, such shrinking of walls should be avoided but cannot be prevented entirely. Generally, wall shrinking does not pose an issue for the dynamic quantities in our system due to the shape matrix formalism. However, it becomes problematic for our energy optimization if wall lengths reach a threshold length on the same order of magnitude as the readjustment of vertex positions during the optimization of the total energy. Then, vertices may slide past each other as depicted in Figure 3.9. Such configurations are topologically entirely valid due to our description of topology via quad-edge. On the other hand, since the energy computation is based on vertex positions, if such a sliding process occurs or vertices get too close, large gradients in the energy are possible, resulting in geometries of intersecting edges as a result of substantial vertex shifting within a single simulation step. Geometrical artifacts, especially, lead to further miscalculations in quantities like the area and respective gradients used for optimization. The area computations, for instance, rely on the shoelace formula, which only produces correct results for polygons that do not self-intersect. Therefore, we submit to removing tiny edges entirely by collapsing the edge's vertices onto the midpoint of the connecting edge if an edge becomes shorter than the threshold of 7.5% of the average edge length. Again, we may derive the new target shape matrices M_0 of the surrounding

cells via their strain once more. Since our descriptions of cells only depend on shape (cf. Figure 3.6A) and not the number of vertices, the difference at this threshold is very minor (cf.⁶⁸). Still, one consequence of such a process is that we remove one degree of freedom from the system.

To avoid this issue entirely, one would have to either include additional terms in the Hamiltonian for the walls to indirectly add a cost to wall shrinking or to apply a wall-based model with a minimal wall length instead of a cell-based model as described in²⁰². Nevertheless, as we will see in the following sections, the overall shape of cells does not matter for the qualitative result during tissue growth, and the shrinking of edges primarily arises in the context of external mechanical constraints, where it still proves not to be the limiting factor.

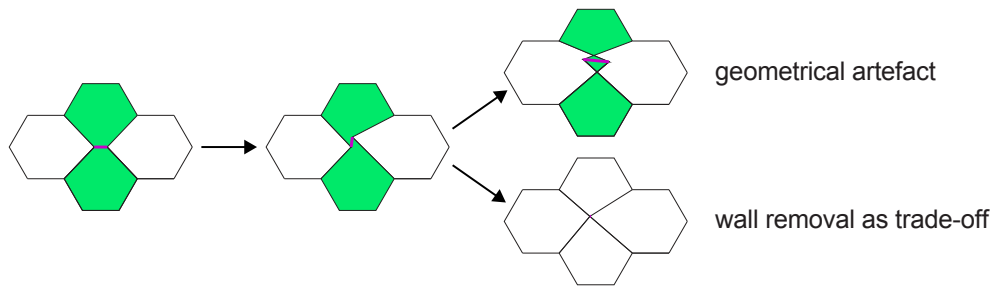


Figure 3.9: Shrinking edges may require removal to ensure numerical stability. During growth and the interaction of multiple cells, cell walls have some potential to shrink, leading to vertices sliding past each other (middle). If ignored, artifacts may arise (right, top). As a trade-off for numerical stability, such walls may be removed, and the corresponding vertices collapsed onto the midpoint of the edge (right, bottom).

3.3.4 Implementation

To implement the theoretical concepts described so far and evaluate the results, we employ a combination of C++, Python, and MATLAB. The implementation of the simulation code, we organize hierarchically. At the core of our approach is the quad-edge data structure¹⁹⁹, for which we use the open-source C++ implementation provided by Heckbert and Bernard²⁰¹. This library is the foundation for our custom C++ "polygeo" package that incorporates all classes and functions from the original C++ quad-edge library while extending its functionality. By separating the original quad-edge library from the enhancements in the C++ surface package, we maintain a clear distinction between the base implementation and our additions. The polygeo package further includes all the geometric analysis tools previously discussed, ultimately integrating and combining the concepts of topology and geometry introduced in Section 3.2. Up to this point, we are also entirely independent of the context studied, and we can apply the implementation of the polygeo package to any type of vertex modeling,

including 3D surfaces. The package provides the geometrical framework for classes and functions introduced in our custom "plantdevelopment" package to compute any quantities related to our mechanical modeling of plant tissues. This package contains everything from the Hamiltonian, growth equations, and anything specific to mechanical modeling, allowing us to run the computations entirely in a C++ environment. In addition, for data evaluation, we wrapped the C++ code in Python 3 with SWIG, a tool for creating an interface between both languages. This interface allows for calling functions implemented in C++ as if they were native Python constructs or even adding new functions within Python, utilizing the underlying C++ code. In this work, we only used the wrapped code in Python in combination with Matplotlib²¹⁰ to simplify the analysis and plotting of the results gained from running simulations in C++. In principle, one could also run simulations directly in Python, only slightly changing the syntax in the transition from C++ to Python. This approach has been applied, for example, already in^{57,68,88}. To access the data comprehensively and (re-) start or continue a simulation from any given step if desired, saving and loading functions have been implemented to save or load the exact mechanical state of a tissue. To generate artificial initial meshes for the simulation, MATLAB, in combination with the wrapped code in Python, was used to exploit its rich repertoire of already implemented functions and for its accessibility. Experimentally derived meshes were created similarly, using processed segmentation data from the ssoftwareMorphographX⁹¹ and PlantSeg²¹¹, as further specified in Section 4. For running the model within C++, we apply the NLOpt package²¹² with its NLOPT_LD_VAR2 algorithm²¹³ to optimize the mechanical energy of the system. Here, we use a local optimization method that allows an efficient optimization process by using gradients for all respective quantities. We update all time-dependent variables for studying growth with a time step Δt . We use the explicit Euler method for integration at each time step, implemented with the GNU Scientific Library (GSL) for C++²¹⁴. Subsequently, all variables and rest configurations are updated, and another optimization and integration step may be performed. The entire procedure is summarized in Figure 3.10. When considering cell divisions or any other changes in the system beyond pure growth, the optimization time increases drastically due to the change of degrees of freedom in the system, extending the search for a new configuration of minimal energy in such scenarios. Because of their performance, the chosen combination of the NLOpt and GSL algorithms enables us to consider tissues, reaching cell numbers up to the same order of magnitude as real-world plant samples. Thus, as the following section will show, we may ultimately use *in planta* data for our *in silico* modeling.

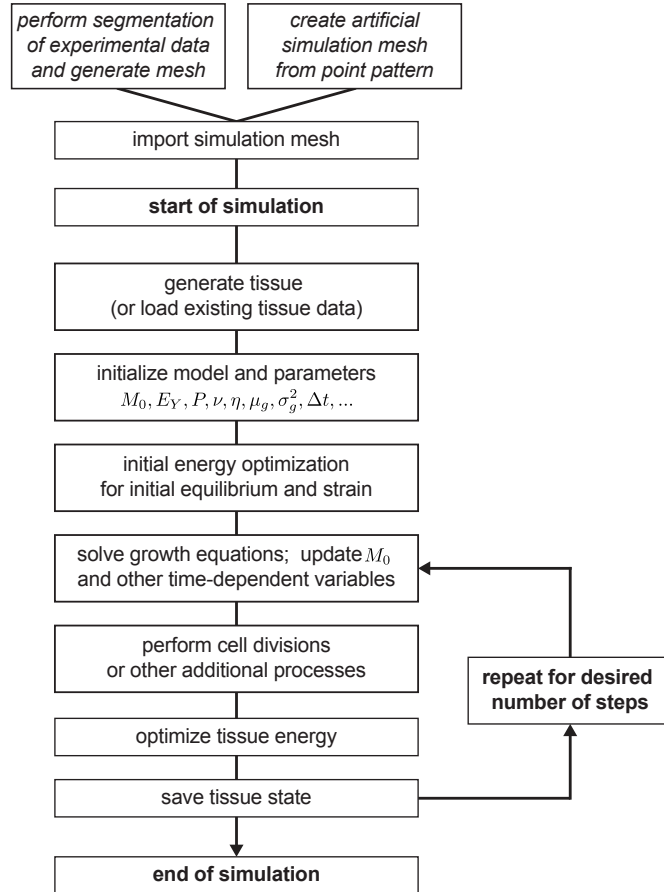
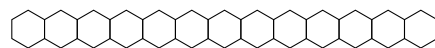


Figure 3.10: Flow chart of the vertex model used in this work. At the very beginning, a simulation mesh must be created by either using experimental data or artificially from scratch. From this mesh, a tissue is either created anew or loaded from a previous simulation. Then, the actual simulation is carried out, including any optional features like cell division, up to the desired number of simulation steps.

4

Modeling radial growth with various tissue geometries and parameters

This chapter explores the framework established in the previous chapter in practice by providing insights into the dynamics of hypocotyl tissue growth based on simulations we run according to the theory presented. We start by describing the process of simulation tissue generation for artificially created meshes and the derivation of meshes using experimental data. For both, we compare different growth modes and explore the mechanical parameters of our model. We also study the effects of changing these parameters on the dynamics of the tissues during growth. Additionally, we introduce the analysis tools we apply to evaluate our results. Ultimately, these basic results allow us to characterize our system and provide insights into possible interactions and correlations between different parameters and growth modes. Therefore, the analyses presented here contribute to the understanding of cause and effect within the mechanical modeling of plant tissues.



4.1 Production of initial meshes

Prior to any simulation, we first require an initial mesh that we can use as a scaffold to simulate cellular growth and division. For this purpose, we developed tools to create such initial meshes based on either only theoretical considerations or experimental data. Here, we will first describe the production of artificial meshes only related to experimental data by choosing their respective size following experimental values. Our general approach throughout this work, no matter the precise procedure to generate a mesh, is to create a Voronoi tessellation,

where each Voronoi cell represents one plant cell. To generate meshes from scratch, in short, we undergo the following steps:

- Create a point pattern
- Compute the Delaunay triangulation of this point pattern
- Compute the Voronoi tessellation, i.e., the dual of the Delaunay triangulation
- Process the Voronoi tessellation to make it suitable for utilization in the simulation

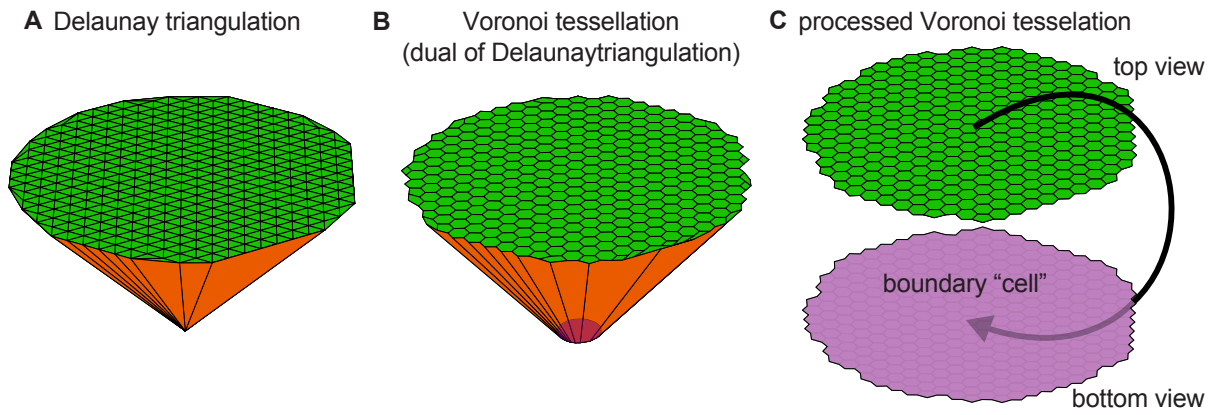


Figure 4.1: Delaunay triangulation allows the generation of Voronoi initial meshes via their dual property. (A) For our initial mesh production, we first produce a Delaunay triangulation from a chosen pattern of points, here resulting in a trigonal mesh (green faces). The addition of one single point below leads to further triangles (orange faces), which simplify the following steps. (B) From computing the dual of the Delaunay triangulation with quad-edge, i.e., switching centroids of faces with junctions, we obtain a three-dimensional Voronoi tessellation of cells (green faces) with some additional excess faces (orange) that need to be removed to flatten the mesh. The purple face at the very bottom is the result of including the extra vertex in panel (A), which we use as a precursor for creating the boundary cell in the next step. (C) Processing the Voronoi tessellation in panel (B) by removing the faces with a z-component leads to the two-dimensional disks we apply in our simulations (top). The bottom of the disk is the boundary cell to close off the mesh topologically. The boundary cell represents the mesh outline as a whole and is adjacent to all cells on the outermost layer (bottom).

We visualize these steps in Figure 4.1. In essence, this means we reverse-engineer our meshes by constructing them indirectly, taking advantage of the connection between Voronoi tessellation and the Delaunay triangulation. This is because they represent each other's dual spaces. Therefore, a mesh of points can easily be transferred from its triangulation into its dual, the Voronoi tessellation, by switching the positions of vertices and centroids of polygons (cf. Figure 4.1A,B). For instance, if we consider a perfect trigonal point pattern, the Delaunay triangulation is a mesh of equilateral triangles. The corresponding dual, the Voronoi tessellation, is a mesh of regular hexagons or honeycomb lattice. By this connection,

we may also directly infer the lattice constant of our Voronoi tessellation. For the ideal case of a trigonal Delaunay triangulation and hexagonal Voronoi tessellation, respectively, the distance from centroid to centroid of each hexagon will be precisely the side length of the equilateral triangles. Thus, choosing a certain lattice constant for the point pattern for the triangulation will immediately transfer to the Voronoi tessellation. We choose this constant accordingly to match by our length units the order of magnitude of typical plant cell size in a young hypocotyl, which is about $a \approx 5 \mu\text{m}$ ^{44,130}. While we are only performing two-dimensional simulations, the quad-edge data structure that provides our topological scaffold, by definition, represents a manifold of genus 0. This means that even a two-dimensional surface, represented by quad-edge, is from a topological point of view a “flattened sphere” (cf. Section 3.2.1). Consequently, beyond the two-dimensional point distribution for the Delaunay triangulation, we add one single point at a z -coordinate below the others (Figure 4.1A) to obtain a three-dimensional triangulation. This is only for practical reasons, though, as it helps the processing of the therefrom constructed Voronoi tessellation. As visualized in Figure 4.1B, the Voronoi tessellation is also three-dimensional, with the top representing the desired mesh and, in addition, excess faces that extend axially. Removing these by applying operations in quad-edge to remove these edges and faces leaves us with only the desired mesh on the top and one single, large cell spanning over the entire boundary (Figure 4.1C). Thus, we will call this “bottom” cell the boundary cell from hereon. As mentioned, we apply this basic procedure to any mesh generation throughout this work.

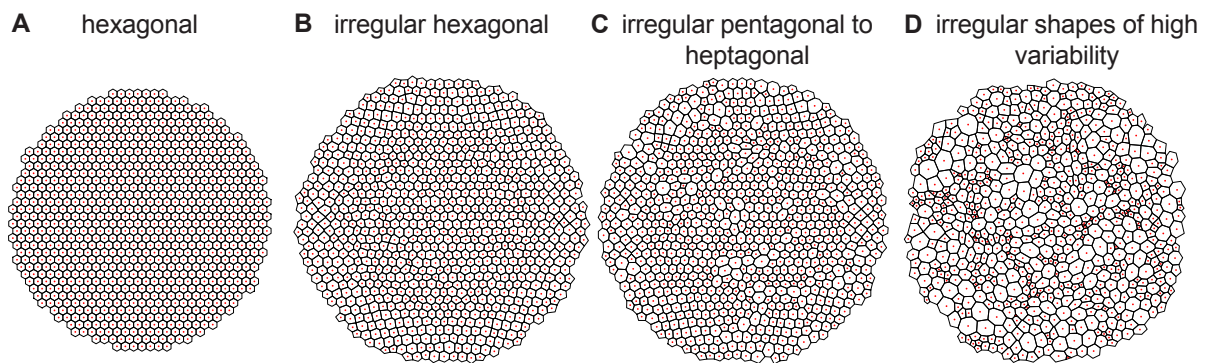


Figure 4.2: Introduction of noise to the Delaunay triangulation allows for various tissue topologies. (A) Regular hexagons as a result of taking the dual of the Delaunay triangulation of a regular trigonal mesh (red dots). (B) Introducing some noise to individual points (red dots) leads to irregular hexagons without changing the tissue topology. (C) Adding higher noise to the Delaunay triangulation when computing the Voronoi tessellation leads to pairs of heptagons and pentagons emerging in the Voronoi meshes. (D) Very high noise in the triangulation results in highly irregular shapes with a wide variety of cell areas and the number of neighbors.

To now create various artificial meshes with this method, we can adapt the point pattern

for the Delaunay triangulation as is displayed in Figure 4.2, where the red dots represent the point pattern that we triangulated and from which we derived the shown Voronoi tessellations. Again, starting with a perfect trigonal mesh, we obtain a regular hexagonal mesh (Figure 4.2A). Introducing some noise into the underlying point pattern (Figure 4.2B) results in retaining hexagons with slight irregularities. Further increasing this noise to the point pattern then leads to the first pairs of heptagons and pentagons to emerge (Figure 4.2C) or for even higher noise, a high level of variability in shapes and anisotropies of the cells (Figure 4.2D). With these meshes, we will perform tissue growth simulations in the following sections to see the influence of variations in the initial conditions regarding geometry and topology.

4.2 Deriving growth modulations for studying differential growth

Differential growth and analysis of experimental data

As discussed in the previous chapters, plant growth is often highly anisotropic. Variations in growth rates across different tissue or organ regions generate directed mechanical forces that shape the structure. These variations in growth rates are commonly referred to as differential growth²⁶. To capture such anisotropic and differential growth in simulations, we need to introduce a source of anisotropy. This can be achieved by modulating either mechanical stiffness (cf. Section 4.3) or the cellular growth by modifying our growth function (Eqn. 5.4) with an additional growth factor κ . Given the limited data on stiffness, we focus in this work on growth rate modulation derived from the experimental data of Zakieva et al.⁴⁴. This dataset provides quantitative measurements of cell positions and areas at different sample ages: 3.5, 4, 4.5, and 5 weeks. Note that while we aim for a cohesive and robust approach here, the following remains a qualitative analysis due to the high variation in biological data and low number of sample tissues.

The tissues we consider here are approximately circular, therefore, we analyze the cell areas A in relation to the radial distances r from the tissue center, i.e., the centroid of the boundary cell (cf. Figure 4.1). We calculate the total radius R of the tissue as the mean of all vertices of the boundary, providing a reasonable approximation and allowing us to define a relative radial position r/R for each cell's centroid. Plotting the binned average of cell areas $\langle A \rangle$ against the relative radii r/R , as shown in Figure 4.3A.(i), reveals a bi-modal distribution with local maxima at both small and large radii and considerable local minima between them. The minimum

corresponds to the continuously dividing cambium stem cells in the hypocotyl, as seen from the analysis of Zakieva et al.⁴⁴. The regions at smaller radii correspond to the xylem part of the tissue, and the regions at larger radii belong to the phloem, as indicated at the top in Figure 4.3A.(i).

The cambium maintains a nearly constant average cell area, while xylem cells expand slowly, and phloem cells expand at a faster rate. This pattern arises because cambium stem cells continuously produce new xylem and phloem cells that rapidly expand, leading to the observed bimodal distribution. The slightly slower progression of the xylem is likely due to its mechanical reinforcement during radial expansion by the deposition of lignin. This gives xylem tissue a much higher stiffness and reduces its potential to expand. Phloem tissue, on the other hand, is much softer and may expand much more. Notably, in very young samples, as a result of the development during primary growth, xylem cells may be similar in size or even bigger than phloem.

To quantitatively describe these area distributions, we approximate the average cell areas $\langle A \rangle$ against the relative radius r/R using a sum of two Gaussian distributions (Figure 4.3A.(ii)):

$$f(x) = a_1 e^{-\frac{(x-\mu_1)^2}{2\sigma_1^2}} + a_2 e^{-\frac{(x-\mu_2)^2}{2\sigma_2^2}}, \quad (4.1)$$

where each Gaussian mainly contributes to one of the local maxima in Figure 4.3A.(i). Note that in contrast to standard normal distributions, we additionally included amplitudes a_i that are independent of the variances σ_i^2 . Although choosing normal distributions to describe the cell areas is somewhat arbitrary, it fits the data well and will provide further benefits, as discussed in the following paragraph. Regarding the goodness of the fits (Figure 4.3A.(ii)), we obtain R^2 values of 0.73, 0.90, 0.91, and 0.72 for tissues aged 3.5, 4, 4.5, and 5 weeks, respectively. Given the biological variance in plant cell areas, these values indicate a reasonable qualitative match. From the fit parameters, we may also extract the locations of the extrema: The relative radii of the maxima $\langle (r/R)_{max,i} \rangle$ are given by the parameters μ_i for each curve and correspond to the centers of the xylem and phloem tissue, respectively. The minima $\langle (r/R)_{min} \rangle$ correspond to the cambium center (dashed lines in Figure 4.3A.(ii)).

4.2. DERIVING GROWTH MODULATIONS FOR STUDYING DIFFERENTIAL GROWTH

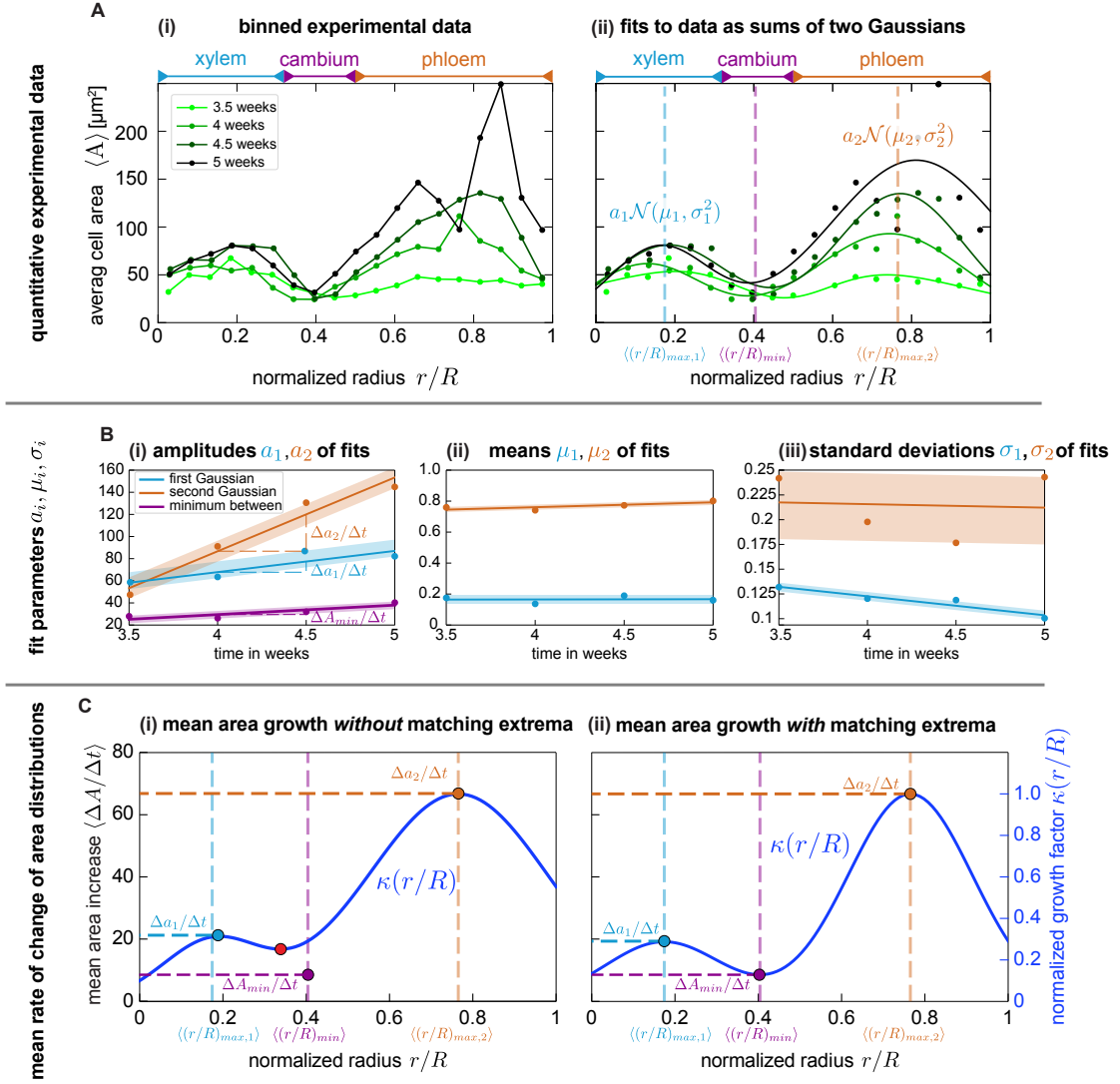


Figure 4.3: Deriving a growth function to replicate differential growth patterns. (A).(i) Binned average area $\langle A \rangle$ of hypocotyl tissues at different ages from 3.5 to 5 weeks (dots) as a function of the relative radius r/R , showing a bi-modal distribution. Data from Alexandra Zakieva⁴⁴; 20 bins. (A).(ii) Fits of sums of two normal distributions of shape $f(x) = a_1 \exp(-(x - \mu_1)^2 / 2\sigma_1^2) + a_2 \exp(-(x - \mu_2)^2 / 2\sigma_2^2)$ to the data in A.(i). Dashed lines represent the relative radii $(r/R)_{\max, \min}$ of the mean extrema positions, considering all four fits. (B).(i)-(iii) Linear fits of parameters of the Gaussians in A.(ii); the amplitudes a_i and the local minimum between, the means μ_i , and the standard deviations σ_i . Lines represent the fit, and shaded areas are the parameter range around the fit. Note that the standard deviations σ_2 vary substantially between individual samples. Slopes for μ_i and σ_i not included to keep visual clarity (cf. Eqn. 4.2). (C).(i) Construction of a radial factor $\kappa(r/R)$ using the difference quotient of the Gaussian fits in A.(ii). Amplitudes a_i are derived from the slopes in B.(i), i.e., $a_i = \Delta a_i / \Delta t$. Means and standard deviations use the mean values of μ_i and σ_i from the fits in A.(ii) in addition to slopes (cf. Eqn. 4.2). The red dot indicates the local minimum. Dashed lines show the average positions and changes of the maxima and the local minimum based on the fits in A.(ii) and the slopes from B. While the maxima coincide, the local minimum is slightly shifted. (C).(ii) Construction of a radial growth factor $\kappa(r/R)$ as in C.(i) but matching all extrema by adapting the variance σ_2 to ensure all extrema, including the local minimum at $\langle (r/R)_{\min} \rangle$ with $\Delta A_{\min} / \Delta t$, coincide with the values, extracted from A.(ii) and B.(i)-(iii). Figure adapted from¹³⁰.

Deriving a radial growth factor to modulate cellular expansion

Before deriving a growth factor for cellular expansion from the experimental data, we establish key constraints to ensure its suitability from both a biological and a numerical point of view. The expression for the growth factor should:

- capture accurately the average rate of change in the cell area for all cell types (xylem, cambium, phloem),
- be transferable to any initial mesh used for the simulation,
- be easy to adapt to allow for high flexibility in its usage,
- be an analytical function that is continuously differentiable.

Since normal distributions meet these criteria, we define the growth factor $\kappa(r/R)$ as a sum of two Gaussians as well^a.

Unlike the area distributions, though, $\kappa(r/R)$ does not represent a static quantity but is intended as a growth *rate*, applied to cells in simulations via our growth function (Eqn. 5.4). To establish a mapping between the mean cell areas $\langle A \rangle$ and the growth factor $\kappa(r/R)$, we exploit that the parameters of the Gaussian fit of the samples between 3.5 and 5 weeks of age in Figure 4.3A.(ii) show a temporal progression. We use this progression to consider a difference quotient $\langle \Delta A / \Delta t \rangle$ for our derivation. We extract the temporal changes of the area distributions from Figure 4.3A.(ii) using linear regressions of amplitudes, means, and standard deviations, which is displayed in Figure 4.3B. The slopes of these fits define our rates of change, and we obtain the parameters, defining $\kappa(r/R)$ as:

$$a_i = \frac{\Delta a_i}{\Delta t} \quad (4.2)$$

$$\mu_i = \bar{\mu}_i + \frac{\Delta \mu_i}{\Delta t} \approx \bar{\mu}_i, \quad \sigma_i = \bar{\sigma}_i + \frac{\Delta \sigma_i}{\Delta t}, \quad (4.3)$$

$$\text{where: } \bar{\mu}_i = \frac{1}{4} \sum_{n=1}^4 \mu_{i,t_n}, \quad \bar{\sigma}_i = \frac{1}{4} \sum_{n=1}^4 \sigma_{i,t_n}, \quad t_n \in \{3.5, 4, 4.5, 5\}, \quad (4.4)$$

where each $i \in (1, 2)$ represents one Gaussian that the growth factor $\kappa(r/R)$ comprises, and where $\frac{\Delta a_i}{\Delta t}$, $\frac{\Delta \mu_i}{\Delta t}$ and $\frac{\Delta \sigma_i}{\Delta t}$ are determined from the slopes of the linear regressions in Figure 4.3B. The mean values $\bar{\mu}_i$ and $\bar{\sigma}_i$ are the means of the values in Figure 4.3B.(ii)-(iii) that were used

^aNote that this approach, using sums of Gaussians, offers very good parameter control, as means μ_i , amplitudes a_i , and standard deviations σ_i define the key features of the curves. Therefore, any derivations and possibly adjustments are much more straightforward than with, for instance, polynomials.

for the regression. Additionally, we may compute the change of the local minimum, similarly to the slopes of the amplitudes, which we denote by $\Delta A_{\min}/\Delta t$ (Figure 4.3B.(i), purple line). Using the parameters obtained from this approach yields the radial growth factor $\kappa(r/R)$, that is displayed in Figure 4.3C.(i). Note that the local minimum with this derivation is shifted slightly toward smaller radii as its position depends on the interaction of the parameters of the Gaussians.

Since we don't expect significant tissue remodeling within the time frame we study in our simulations (cf. Zakieva et al.⁴⁴), we assume the extrema to remain in place, both regarding their relative radial position r/R and the mean changes of amplitudes. Thus, we discard the shifts to the maxima, i.e., assume $\mu_i = \bar{\mu}_i$ and align the cambium minimum with the area rate of change of older samples, $\Delta A_{\min}/\Delta t$. We approach this by adjusting the standard deviation σ_2 because it substantially fluctuates without any clear pattern, making it a reasonable parameter to adapt. This yields the final growth factor, where now all extrema match their expected position (Figure 4.3C.(ii)). We normalize $\kappa(r/R)$ to its maximum value, ensuring a modulation range of $0.1 \leq \kappa(r/R) \leq 1$.

Simulation parameters, growth conditions, and evaluated quantities

To set the basis for the simulation results grounded on the concepts discussed so far, we briefly summarize the parameters used in this work along with the reasoning behind them to a set of "standard" parameters and conditions. We apply these throughout the rest of our study unless stated otherwise because we primarily introduce anisotropies in growth through the radial growth factor discussed earlier. The following parameters apply to all cells in the tissue:

Parameter	Value
turgor pressure P	0.5 MPa
Young's modulus E_0	300 MPa
Poisson's ratio ν	0.3
mechanical feedback parameter η	0.01
baseline growth rate μ_g	75
baseline growth rate variance σ_g^2	25
simulation time step Δt	0.01

Table 4.1: Simulation parameters

The first three parameters in table 4.1, the mechanical properties of the cells, are chosen according to recent literature estimates^{46,66,85}. The mechanical feedback parameter is chosen

such that mechanical feedback does have a clear influence on the dynamics of growth but does not overshoot as previous works have shown too high feedback to cause even more anisotropic growth, thus leading to the opposite effect by the overreaction to mechanical cues⁶⁷. The baseline growth rate and variance, on the other hand, represent an overall modulation for cellular growth as a whole by drawing random numbers from a normal distribution $\mathcal{N}(g, \sigma_g^2)$ in each simulation step to ensure stochastic growth. Since this is applied to all cells equally, it does not introduce any distinct modulation of growth over time. The mean g and variance σ_g^2 values are chosen to suit the other simulation parameters. In our simulations, cell sizes in our meshes closely link to experimental data, meaning cell diameters of about $5\mu\text{m}$ translate to 5 length units in the simulations to which all size-dependent parameters are adapted. Also, at the beginning of the simulation, we initially set the target shapes M_0 as the current targets M . In the case of perfect hexagons, we require a minor perturbation. This perturbation may be introduced by slightly altering the target shape M_0 or by applying a small random offset to each vertex's coordinates. Here, we choose the latter and apply a random shift to the vertex positions, averaging to 7% of the hexagonal edge length. This offset introduces some initial strain via the first energy optimization, during which the vertex positions are updated. Without this strain, there would not be any growth, and the system would remain in a state resembling an unstable equilibrium because the vertex positions are already optimal. Finally, when initializing all simulation parameters, the turgor pressure P and Young's modulus E_0 of the boundary cell are set to zero because it only serves the role of topologically closing off the tissue. Thus, we ensure the boundary cell is entirely passive and does not contribute to the Hamiltonian.

When running simulations of growth and cell divisions, beyond presenting the qualitative features of the resulting meshes, we will briefly discuss a few quantitative analyses, giving us a better insight into cause and effect regarding our simulations' input parameters and outcomes. When our tissues grow, the cell areas will change, of course. Thus, we will continue to monitor to average cell area $\langle A \rangle$ w.r.t. the normalized radius r/R . The normalized radius will also act as the reference for all other quantities we evaluate. These include the average cell stress angles $\theta(\vec{\sigma}_{max})$, which we will use to analyze any stress patterning on the tissues. The cell stress angle we define using the direction of maximum stress that we obtain from performing an eigendecomposition of the stress tensor σ . The direction of maximum stress is the eigenvector corresponding to the stress tensor's largest eigenvalue. As we are considering a radially symmetric tissue that grows outwards from the tissue center, we do not take the x-axis as a reference to express the direction of the maximum stress by an angle. Instead, we use the tissue center as a reference point to define an angle from this direction. From

the tissue center, we consider the radius vector $\vec{r}$ pointing to the centroid of a specific cell we consider. For this cell, we compute the direction of maximum stress with respect to the cell's centroid. The stress angle $\theta(\vec{\sigma}_{max})$ is then the angle between the cell's centroid's radius vector and the cell's direction of maximum stress, as visualized in Figure 4.4A. Thus, if the angle is, for instance, 0° , it means the maximum stress points radially outwards with respect to the tissue center, and we call this radial stress. If the angle is 90° with respect to the tissue center, we call this circumferential stress. These terms are also illustrated in Figure 4.4A, where the black lines inside cells display the direction of maximum stress. Additionally, we also color code the orientation of stress. Circumferential stress, i.e., stress normal to the radius vector from the tissue center to a cell, we represent by a blue fill, and radial stress parallel to the radius vector, we represent by a red fill. In between those states, we have a color gradient for the transition. Also, in each cell, we display the relative magnitude of stress anisotropy, bounded between values of 0 and 1. This is the ratio of the eigenvalues ω_i of the stress tensor σ , given by $|(\omega_1 - \omega_2)|/(\omega_1 + \omega_2)$. We display this anisotropy by the opacity of the fill and the length of the black lines, representing the direction of maximum stress.

Beyond the orientations of stresses in cells, we also measure the total stress experienced by a cell by computing the Frobenius norm of stress, i.e.

$$\|\sigma\|_F := \sqrt{\sum_{i=1}^m \sum_{j=1}^n |\sigma_{ij}|^2} = \sqrt{\sigma_{11}^2 + \sigma_{22}^2 + 2 \cdot \sigma_{12}^2}, \quad (4.5)$$

the square root of the sum of all matrix entries multiplied by themselves, which can be considered a measure of the contribution of stress to the mechanical energy of the tissue. For all of the quantities mentioned before, we consider binned averages, where each bin represents 5% of the tissue radius over which cells' properties are averaged.

Equipped with all of this, we now look into the simulation results of this work.

4.2.1 Simulation of hexagonal tissues with uniform and modulated growth

With the initial conditions mentioned previously, we aim to perform growth simulations for various tissues, where we intend to gradually increase complexity. We begin with a fully hexagonal mesh to compare two growth modes: uniform growth and growth modulation by the radial growth factor $\kappa(r/R)$, which we derived in the previous section.

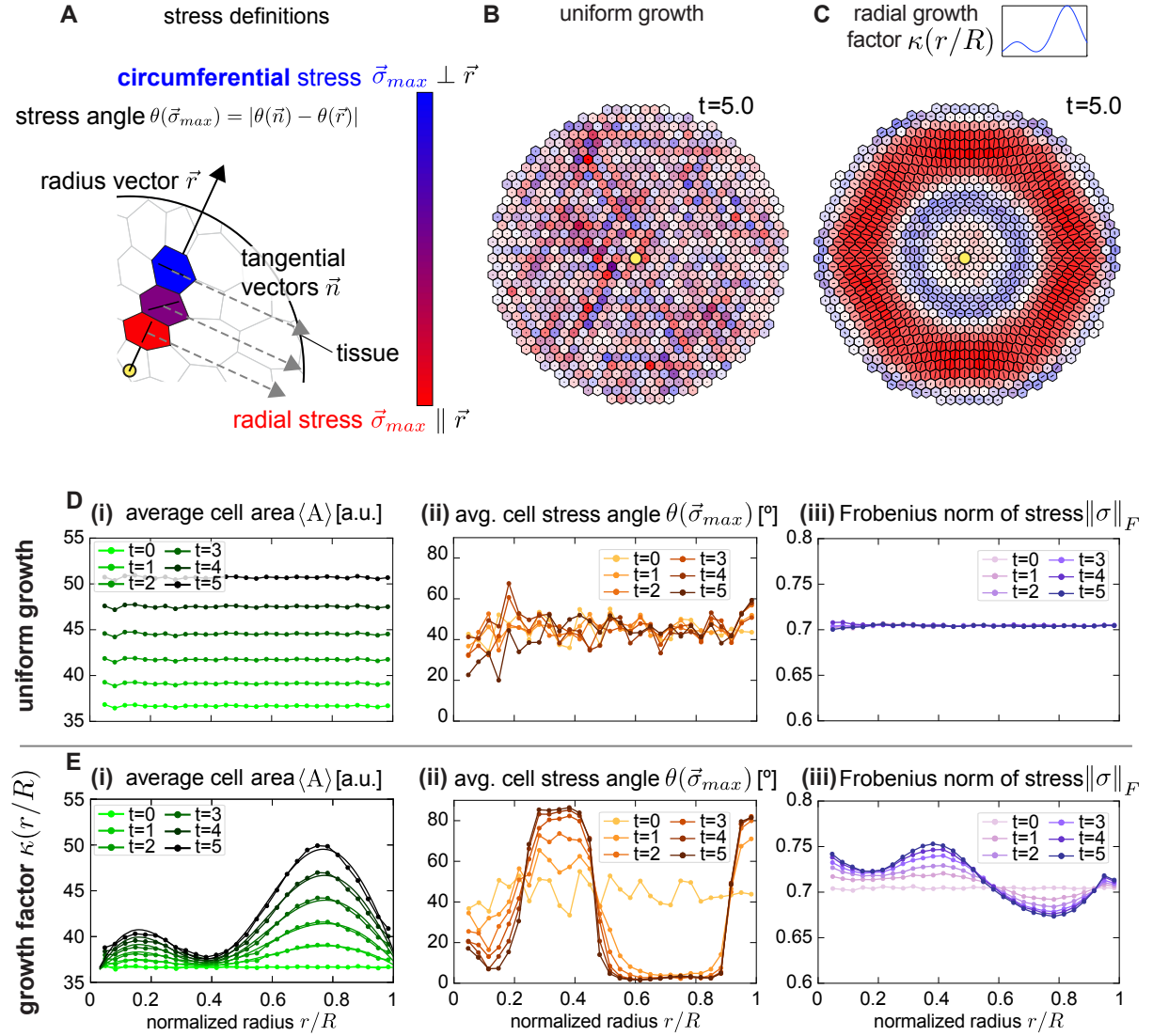


Figure 4.4: Simulation of tissue growth requires anisotropies to create patterning (A) Definition of the radial stress and circumferential stress, as well as the stress angle $\theta(\vec{\sigma}_{max})$. Measures always use the tissue center as a point of reference. The color bar captures the variation in stress between the extreme cases of the vector of maximum stress $\vec{\sigma}_{max}$ having either only a component in radial ($\vec{r}$) or circumferential ($\vec{n}$) direction. Panel and caption adapted from¹³⁰. (B) Growing a hexagonal tissue for 500 simulation steps with a uniform growth pattern and mechanically isotropic parameters does not produce any distinct patterning. (C) Applying the radial growth factor $\kappa(r/R)$ (small inset, also shown in 4.3), as a modulation to tissue growth, results in a distinct stress pattern of concentric rings to emerge during the simulated 500 steps with large circumferential stress (blue) in the region that represents the cambium and radial stress (red) in the regions representing xylem inwards and phloem outwards. (D).(i)-(iii) Quantitative analysis of uniform growth of the hexagonal tissue shown in (B) by considering area distributions D.(i), stress orientations D.(ii) and total stress D.(iii) with respect to the normalized radius r/R at different simulation time steps from $t = 0$ (initial mesh) to $t = 5$ (500 steps). For this growth mode, the distributions of these quantities do not largely change over time. (E).(i)-(iii) Same analysis as in D.(i)-(ii) for the hexagonal tissue with the radial growth factor $\kappa(r/R)$ (Figure 4.3C.(ii)). The clear stress pattern visible in C.(i)-(ii) that emerges from the radial growth factor is reflected quantitatively by modulations of the area E.(i), stress orientation E.(ii), and stress magnitude E.(iii), where low local growth results in constraints of tissue growth, resulting in large circumferential stress. The overall stress is only released at large radii, in the radial range with the highest growth rate.

We grow the initial mesh shown in Figure 4.2A for 500 time steps of size $\Delta t = 0.01$ to compare both growth modes, uniform growth and modulated growth at time $t = 5.0$. Already, by looking at the color-coded meshes after growth in Figure 4.4B,C, the difference is quite striking. For uniform growth, there is no stress pattern at all.

In contrast, for the tissue grown with the radial growth factor $\kappa(r/R)$, we observe distinct concentric rings of circumferential and radial stresses arising that correspond very much to the curve of the applied growth factor. This behavior can also be seen quantitatively by an analysis of the resulting area distributions (Figure 4.4D.(i) and E.(i)). Cells do not show any modulation of their size for uniform growth, and the area distribution remains uniform while the tissue continuously grows. Applying the radial growth factor, as intended, the cambium grows very little; the xylem and especially the phloem have the most significant contribution to the overall tissue area increase. This pattern is also reflected in the orientations of maximum stress (Figure 4.4D.(ii) and E.(ii)). In both cases, there is no stress pattern at the beginning of the simulation ($t = 0$). While for the uniform growth, it remains that way, as also visible from the tissue after growth (Figure 4.4B), for the simulation with the radial growth factor, there is an apparent emergence of stress patterning already after a few simulations steps at $t < 1.0$ that imprints a concentric pattern of radial and circumferential stresses. Note that the stress angles with respect to the relative radius (Figure 4.4E.(ii)) change very drastically between $t = 0$ and $t = 1$. In contrast, the pattern becomes stable later, with stress orientations in the xylem and phloem approaching 0° and in cambium 90° . Already between $t = 4$ and $t = 5$, there are only minor changes, which is why for the upcoming simulations, we will typically simulate until $t = 5$ and perspective use tissue states at $t = 5$ as starting points for studying cell divisions in chapter 5.

Beyond the distributions of cell areas and maximum stress angles, also the total magnitudes of stress (Figure 4.4D.(iii) and E.(iii)) show a clear difference between the different growth modes and the thus introduced anisotropy of cellular growth by the growth factor. While for both cases, the total stress, measured by the Frobenius norm, is of the same order of magnitude, for uniform growth, it vastly does not change throughout the simulation. For a modulation with the radial growth factor, though, we observe a modulation where we may conclude total stress to rise in cells that grow slower than their surroundings, i.e., especially in the cambium around $r/R \approx 0.4$. This observation suggests that the cambium tissue is subject to forces from the xylem and phloem as both grow faster, pushing against or providing constraints to the expansion of the slower-growing cambium. This effect also occurs at the tissue center and

the boundary layer of cells, where the growth function decays to zero. At the boundary, the slower growth limits the overall outward expansion of the tissue, which is also reflected by circumferential stress at the boundary. At the center of the tissue, one might expect to observe circumferential stress as well; however, because the center represents a singularity where the circumferential direction is undefined, the stress field becomes isotropic instead. Still, we can see the compressive effect on the tissue center from the surrounding cells by the fact that the Frobenius norm of stress in cells increases at the very center of the tissue, the cambium, and the boundary (local maxima in Figure 4.4E.(iii)). Supplemental Figure B.1 shows the same plots as in Figure 4.4E with the growth factor $\kappa(r/R)$ as an overlay over these panels where one may observe the link between the growth factor and the analyzed tissue characteristics.

In conclusion, uniform and modulated growth results for the hexagonal mesh allow us to summarize: uniform growth does not produce any stress pattern or differential growth. Consequently, we require some level of anisotropy in our simulation, such as a radial growth factor to modulate growth. For these two growth modes, already the tissues, as visualized in Figure 4.4B,C, provide a qualitative picture of the effect of the growth modulation. For the specific growth factor we derived, circumferential stress emerges, especially in the cambium section of the tissue and at the boundary cell layers. This stress response results from the reduced growth rate in these regions, causing the faster-growing tissues to exert additional stress on the slower-growing ones. One thing to point out is that if we look closely at the final mesh in Figure 4.4C, we may observe slight differences in the magnitudes of radial stress at large radii (see the slight variation in the shading of red) despite the uniformity of the tissue. While these differences only seem minor, they coincide with the hexagonal geometry of the cells. Hence, we consider this to be an artifact from the highly regular tissue structure. Consequently, it is highly desirable to proceed with alternatives for initial meshes with a less regular cell arrangement, thus not leading to such artifacts. While we might use other artificially created, less regular meshes, we take our chances here to derive initial meshes directly from experimental data to obtain more realistic results. We discuss this derivation based on experimental data in the following section.

4.2.2 Deriving tissues and growth modulations, based on experimental data

Creating initial meshes from experimental data

To increase the realism of our simulations beyond the dynamics we study, we aim to improve our initial conditions as well by deriving meshes based on experimental data. Therefore,

we established a pipeline for creating simulation meshes from microscope images of lateral cross-sections of young hypocotyls, such as a two-week-old sample, displayed in Figure 4.5A by a microscope image. From this sample, we also derive a simulation mesh for our further simulations from this point onward. The experimental data in this context was collected by Xiaomin Liu¹³⁰, who also performed the segmentation of the microscope image as a first step (cf. Figure 5.6D). She applied the tools PlantSeg²¹¹ and MorphographX⁹¹ with the procedures that Alexandra Zakieva established in her earlier work⁴⁴. With these tools, we obtain the segmentation of the tissue, from which we then extract the centroid positions of the cells. These centroids we use for our already described approach to construct a Voronoi mesh off this data (cf. Section 4.1). As a result, we obtain the Voronoi mesh (Figure 4.5B), corresponding to the segmentation of the microscopy image. We chose to stick to Voronoi tessellations here instead of taking the segmentation of the tissue directly for multiple reasons. First, and most importantly, the segmentation contains many vertices for each cell's outline, not only the junctions between cells but also along the walls. Thus, a large number of vertices have to be considered during the optimization process. Using a Voronoi tessellation decreases this number from about 25.000 to about 1.800. Since computation time increases roughly in proportion to the number of vertices — each of which must be optimized at every simulation step — using the full segmentation would have exceeded our computational resources. Therefore, we needed to minimize the number of vertices to a manageable order of magnitude, which in practice turned out to be of order $\mathcal{N}_\alpha = 10^3$. Beyond the sheer number of vertices to consider, some of the cells contain tails or concave shapes, which are also challenging for the numerical stability of our simulations. Consequently, we require a trade-off between numerical stability and accuracy of the simulated geometries. While some of the details of the original segmentation are lost this way, the main features, such as the cell area distribution, are preserved (cf. Figure 4.6B), and there is only a slight difference in the area distributions between the segmentation and its corresponding Voronoi mesh. Beyond the apparent differences in geometry and topology, this coarse-grained approach also represents an opportunity to see if specific cell arrangements that are present in the segmentation can be recreated by growth and or cell division from the Voronoi tessellations as well, i.e., if we can recreate topological and geometrical conformation to the segmentation as emergent behavior in such tissues.

By choosing this coarse-graining, we also assume, at least to some extent, that the dynamics are independent of the exact mesh we use for our simulations. Our theoretical considerations of topology and geometry, in principle, predict the independence of individual cells towards

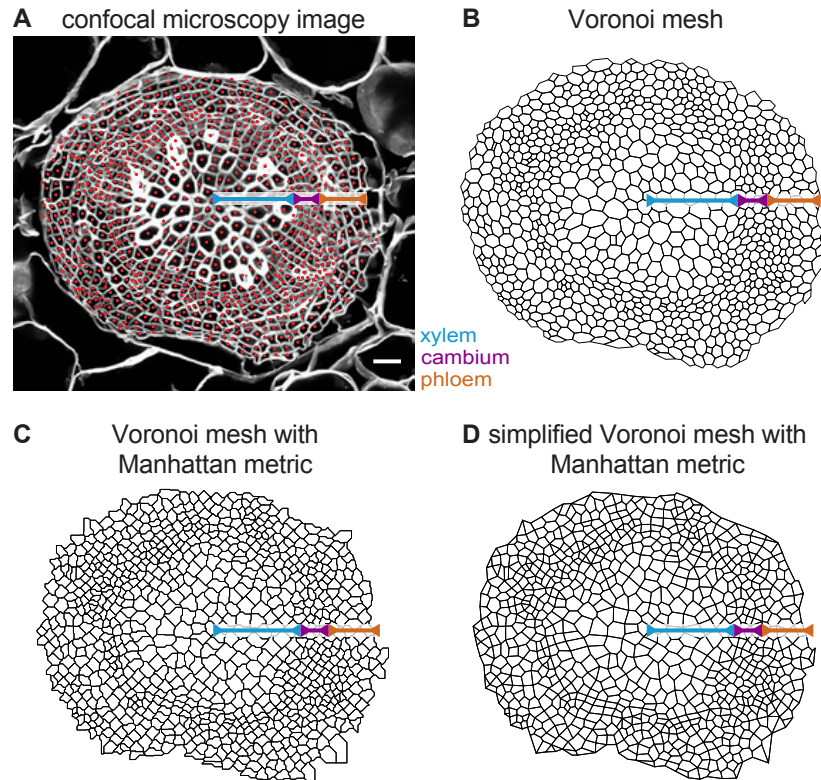


Figure 4.5: Creation of realistic meshes involves microscopy images and image segmentation.

(A) Microscope image of a young hypocotyl of the age of two weeks. Bright, large cells towards the center are xylem cells, surrounded by cambium stem cells and phloem tissue (blue, purple, and orange bars). Huge cells at the boundary of the image are cortex cells that ultimately detach from the organism. Scale bar, 15 μm (B) Voronoi mesh, generated from the centroids (red dots in (A)) of segmented cells with the procedure shown in Figure 4.1, retaining quantitative properties (cf. Figure 4.6B) while providing a simplification regarding the number of vertices and numerical robustness. Note that by our construction of a Voronoi mesh, the outermost cell layer of the segmentation is lost during mesh creation to create the boundary. (C) Generation of a Voronoi mesh, using the same data and procedure as in (B), but with Manhattan metric instead of Euclidean distance. (D) Simplification of the Manhattan Voronoi mesh shown in (C) in order to avoid two-way junctions and tails to improve numerical feasibility. Figure adapted from¹³⁰.

the number of vertices and edges that define it due to the independence of the shape matrix M to these. Nevertheless, in a setting with neighboring cells in a tissue, such independence is not to be taken for granted due to cell interactions. Therefore, we will ultimately challenge this independence by comparing simulation results with various meshes, including different kinds of fully artificially created meshes and experimentally derived meshes. Beyond the already mentioned Voronoi tessellation, based on experimental data, we also consider the Voronoi tessellation, assuming a Manhattan metric. This means that instead of considering an Euclidean distance, we construct the Voronoi mesh with a Manhattan distance, also called the taxicab metric. Thus, the distance between two points $p_1 = (x_1, y_1)$ and $p_2 = (x_2, y_2)$ is not like

in the Euclidean distance $\Delta p = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$, but instead $\Delta p = |x_2 - x_1| + |y_2 - y_1|$. The resulting mesh is shown in Figure 4.5C. The advantage of this mesh is that some features of the original segmentation are retained better than those of the Voronoi tessellation with Euclidean distance. For instance, cells tend to have more rectangular shapes instead of being more isotropic and round. The change in shapes is especially noticeable for cells in the cambium section of the tissue (purple radius range in Figure 4.5), where in the experimental data, cells are typically forming files of rectangular cells, stacked on top of each other as a result of their bi-directional division. This symmetry is also present in the Manhattan metric Voronoi tessellation. As with directly using the segmentation, one challenge for numerical simulations is again the occurrence of tails and short edges, rendering it almost impossible to achieve a stable and robust simulation. Thus, we further simplified the Manhattan metric Voronoi mesh by removing tiny edges and all two-way junctions, i.e., flattening the cell walls to reduce complexity in favor of numerical stability. The final result of this approach is displayed in Figure 4.5D, which, again, retains the higher level features such as area distribution but also still features more rectangular cells, especially in the cambium area (purple radius range in Figure 4.5).

In the following, we compare the standard Voronoi tessellation and the Manhattan metric one, in addition to a variety of artificially created simulation meshes, in view of the robustness of our simulation results, using these different meshes.

Deriving a growth factor for initial meshes, based on experimental data

Because we base the Voronoi meshes on a real-world tissue that has grown for two weeks, their cell area distributions are non-uniform, unlike the initial assumption of uniform hexagons in the previous section of this chapter. Consequently, the radial growth factor, $\kappa(r/R)$, must be adjusted to the new mesh. To recapitulate, in our earlier derivation, we assumed that tissue growth began with a uniform cell area distribution and that subsequent growth would reproduce the experimentally observed distribution. Our goal remains to simulate tissue growth that leads to the evolving area distribution matching the observed one in older samples (ages between 3.5 and 5 weeks; see Figure 4.3A.(i)). Therefore, it is important to acknowledge the non-uniform distribution that is already present in the two-week-old sample (Figure 4.5A) and adapt the growth function accordingly. For this reason, we compare the experimental data of average cell areas $\langle A \rangle$ along the relative tissue radius r/R for meshes at the ages between 3.5 and 5 weeks and for the 2-week-old sample. Both area distributions - as fits to the data by sums of two Gaussians - are displayed in Figure 4.6A,B. The most striking difference between

both is that in contrast to the more mature samples, in the 2-week-old sample, the xylem cells are larger in area than the phloem cells. In the later developmental stages, starting at around 3.5 weeks, the opposite is the case. This deviation is mainly because of two reasons: On the one hand, studies have shown a very high biological variation in ratios of xylem and phloem and even in the total size of the entire hypocotyl¹¹⁷. On the other hand, beyond this variety in the area distributions, the hypocotyl also undergoes substantial remodeling between the very early growth stages, subsequently to its primary elongation growth, until maturity and its inflorescence. Consequently, the area distribution of the two-week-old sample has to be considered for deriving a radial growth factor $\kappa(r/R)$.

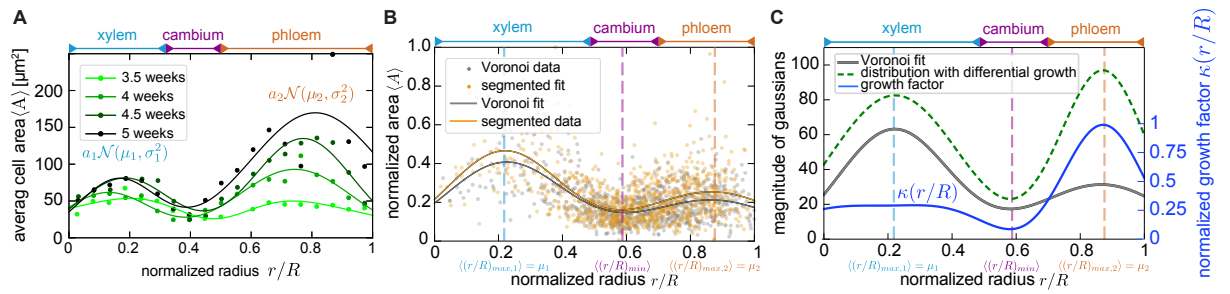


Figure 4.6: Derivation of a growth function for the tissue shown in Figure 4.5A. (A) Fits of sums of two normal distributions, defined as $f(x) = a_1 \exp(-(x - \mu_1)^2 / 2\sigma_1^2) + a_2 \exp(-(x - \mu_2)^2 / 2\sigma_2^2)$ to binned experimental data of hypocotyl tissues at ages from 3.5 to 5 weeks (dots), plotted against the relative radius r/R . Data from Alexandra Zakieva⁴⁴. (B) Comparison of cell area distributions from two different methods: segmentation of a microscope image Figure 4.5A) and a Voronoi mesh generated from a two-week-old tissue sample (Figure 4.5B). The areas are normalized by their maximum values. Both datasets are fitted using the sum of two normal distributions as in (A) and show a close agreement between the segmentation and the Voronoi mesh. Dashed lines indicate the relative radii $r/R_{\max, \min}$ corresponding to the extrema in the Voronoi data. (C) Radial growth factor $\kappa(r/R)$, derived using the same approach as in Figure 4.3. The Gaussian parameters μ_i and σ_i are taken from the fit to the Voronoi mesh (B). The amplitudes a_i are determined based on the previously established amplitude slopes (Figure 4.3B). To ensure that the extrema positions $r/R_{\max, \min}$ remain unchanged, we adjust σ_i accordingly (cf. Figure 4.3C). This results in the (normalized) growth factor $\kappa(r/R)$ (blue line). By adding the unnormalized growth factor values to the Voronoi fit (black line), we estimate how the tissue area distribution will eventually change through differential growth within a week of growth (green dashed line). Figure adapted from¹³⁰.

We follow the same approach as previously described for its derivation, assuming also that the global tissue structure remains mostly unchanged over the simulated time scales while still accounting for the initial area distribution that the two-week-old sample possesses. The primary difference is now that for the means and standard deviations, i.e., μ_i and σ_i , we do not use the mean values from the fits of the data between 3.5 to 5 weeks anymore (cf. Eqn.4.3), but

rather the parameters of the two-week-old sample (Figure 4.6B). That leads to the parameters:

$$a_i = a_{i,2weeks} + \frac{\Delta a_i}{\Delta t}, \quad (4.6)$$

$$\mu_i = \mu_{i,2weeks}, \quad (4.7)$$

$$\sigma_i = \sigma_{i,2weeks} + \frac{\Delta \sigma_i}{\Delta t}, \quad (4.8)$$

where the terms $\frac{\Delta a_i}{\Delta t}$ and $\frac{\Delta \sigma_i}{\Delta t}$ are the same as in the previous derivation (cf. Eqns. 4.2,4.3), but the overall reference is now the area distribution of the two-week-old sample, i.e., $a_{i,2weeks}$, $\mu_{i,2weeks}$, and $\sigma_{i,2weeks}$. Retaining the positions of all extrema as before (dashed horizontal and vertical lines in Figure 4.6C), by slightly adapting the standard deviations of the Gaussians, results in the emerging area distribution, assuming differential growth for the two-week-old tissue (Figure 4.6C, black line and green dashed line). The radial growth factor $\kappa(r/R)$ is then the difference between the distribution at 2 weeks and this prediction (blue line in Figure 4.6C). As before, growth in the xylem and cambium sections of the tissue remains low, whereas it is substantially faster in the phloem.

With this new growth factor $\kappa(r/R)$ that is specifically adapted to match the Voronoi tessellation of the segmented tissue at 2 weeks of age, we now perform further simulations to get a better understanding of our system, its robustness, and its response to alterations of the mechanical environment.

4.3 Simulating growth with different mechanical properties

4.3.1 Influences of tissue structure on stress patterning

Now, with all the tools for simulating and analyzing the growth of the experimentally derived tissue, we intend to get a better understanding of the dynamics of our system and its response under variations of the mechanical parameters. For this purpose, we compare the robustness of pattern formation for different initial meshes. Additionally, we explore different simulation parameters and the resulting dynamics from them, including the stiffness, i.e., Young's modulus E_0 , turgor pressure P , and Poisson's ratio ν .

We begin with studying the robustness of the stress patterning. Previously, we had observed stress pattern formation from implementing a radial growth factor $\kappa(r/R)$ to the growth of a

hexagonal mesh (Figure 4.4C). This is because the growth factor provides a source of anisotropy and, thus, a mechanical conflict, leading to patterns of concentric rings.

Here, we apply the adapted radial growth factor from Figure 4.6C, which we derived specifically for the Voronoi mesh, based on the two-week-old experimental sample. This is also the one we will use in all simulations from this point onward. In principle, we must derive a separate growth function for every tissue, matching the respective area distribution to account for the correct growth modulation in all xylem, cambium, and phloem. However, to qualitatively compare the robustness of those patterns in this section, we stick to this afore-

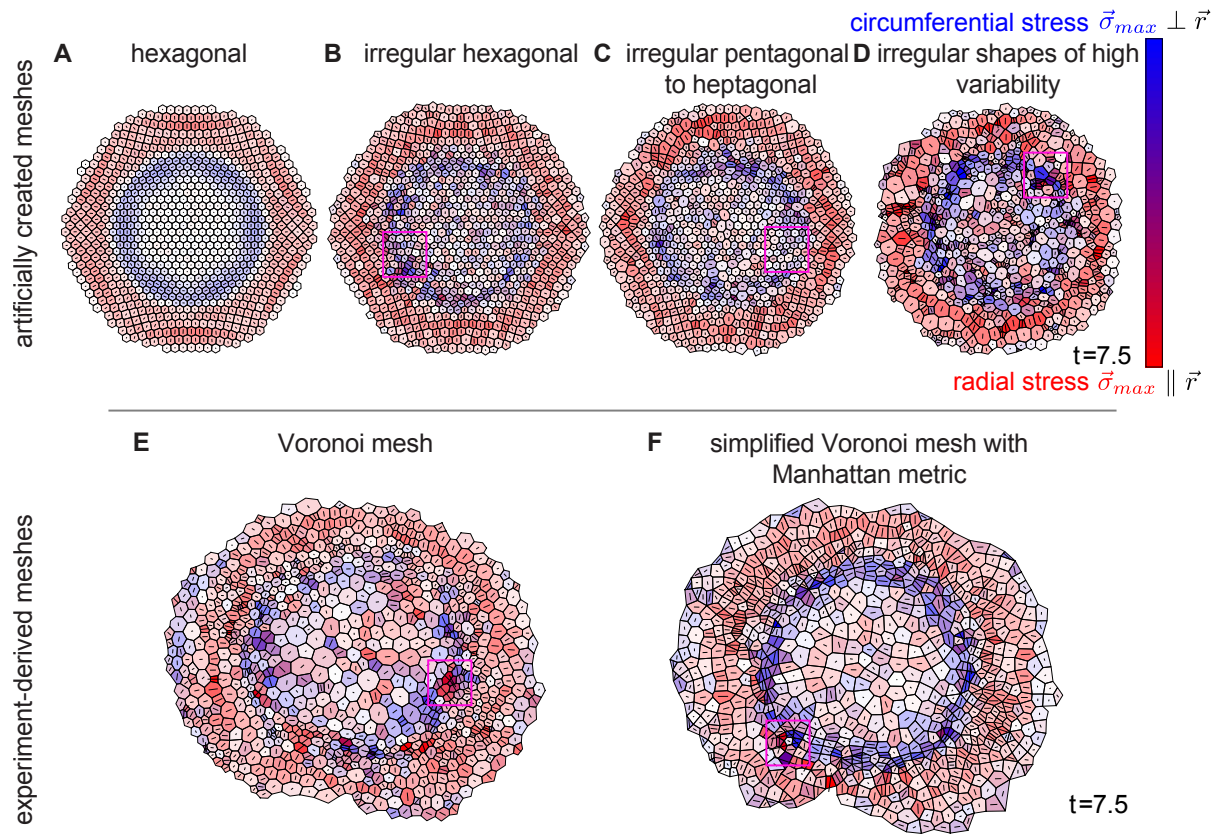


Figure 4.7: Mechanical stress patterns from differential growth are robust even against very different tissue topologies and geometries. (A-D) Simulations of growth, modulated by the previously derived radial growth factor $\kappa(r/R)$ (Figure 4.6), with artificially produced meshes as in Figure 4.2). From initially uniform hexagons to shapes of wide geometrical and topological variety, we observe large local stress anisotropies only for highly irregular meshes (D). In all cases, though, the global stress pattern, observed especially in more regular tissues, is retained. **(E,F)** Experimentally-derived meshes (Figure 4.5B,D) under radial growth with growth factor $\kappa(r/R)$. Both the Voronoi mesh with Euclidean distance (E) and with Manhattan distance (F) show a distinct concentric stress pattern. Consequently, the resulting stress pattern is robust against all initial mesh geometries and topologies investigated here, indicating high circumferential stress in the vascular cambium (blue ring) due to being sandwiched between the faster-growing xylem and phloem tissue. Boxes highlight areas of minor local perturbations. Panels E,F adapted from¹³⁰.

mentioned growth factor. We apply it to both artificially created meshes of varying complexity and uniformity (cf. Figure 4.2), and the experimentally derived Voronoi tessellations with either Euclidean or Manhattan distance. The results for the growth of these, until $t = 7.5$, are displayed in Figure 4.7. The upper parts of panels (A-D) represent meshes created from scratch and which are based on trigonal point patterns. From left to right, these represent hexagonal Voronoi meshes with increasing noise. The bottom panels of (E-F) represent the Voronoi mesh, derived from the image segmentation of the two-week-old hypocotyl sample and the construction of the Voronoi mesh using the Euclidean distance (E) or the Manhattan distance (F). As we may see, the overall pattern that we observed before is present in all meshes used. Differences in the pattern are typically minor or very localized interruptions of the concentric rings, highlighted by the pink boxes. Notably, no deviation from the pattern is apparent due to the absence of any irregularities in the hexagonal mesh (panel A).

The fact that the pattern remains clearly in place with all meshes shows the robustness of the pattern formation and its overall independence on the exact mesh. Nevertheless, in detail, there are many differences, especially for large anisotropies in the meshes, as in panel D, high radial and circumferential stresses occur by local mechanical conflicts, especially between neighboring cells of very different sizes.

While the Manhattan Voronoi tessellation (panel F) seems particularly realistic in comparison with the image segmentation and already retains some of the geometry, especially in the cambium tissue, the way we create it contains some levels of arbitrariness by the simplifications in the tissue topology and geometry we made (cf. Section 4.2.2). Thus, the Voronoi mesh with Euclidean distance is our choice to continue with, especially in light of cell division that we will consider in Chapter 5 to analyze if we may observe similar tissue structures to the ones from the segmentation as emerging behavior rather than already having it imprinted like on the Manhattan Voronoi tessellation. In the following we will further explore the effect of variations in the mechanical parameters of cells to understand the dynamics and patterning as a consequence of the interplay of cell and tissue mechanics.

4.3.2 Variations of Young's modulus

Next, we aim to demonstrate the effect of modulating not only the growth factor $\kappa(r/R)$ as a function of the relative radius r/R but also changing the stiffness of cells. Typically, in our simulations, we applied a reference stiffness of $E_0 = 300$ MPa for all cells, regardless of whether they correspond to phloem, cambium, or xylem. We apply this generalization because of the absence of reliable measurements, especially in combination with time-dependent effects like

the placement of new cell walls during cell division. Still, we analyze the effects of alterations in stiffness. We again consider the cambium tissue. In our previous simulations, we saw a circumferential stress pattern emerge from the reduced growth there due to applying the radial growth factor $\kappa(r/R)$. In the cambium, the resulting growth factor is in the range of half to a quarter compared to the xylem tissue. Besides this modulation, growth substantially depends on the stiffness. A large stiffness for a cell leads to a higher energy cost to deform and grow compared to a cell of lower stiffness. In the following, we consider how our stress pattern changes if we adapt the stiffness of cambium cells, i.e., cells around $r/R_{min} \pm 7.5\%$ of the local minimum of the cell area distribution in the tissue. We compare stiffnesses that are merely 25% of the reference stiffness and stiffnesses of five times the reference value of $E_0 = 300$ MPa. To further illustrate the effect on the change of stiffnesses here, we compare simulations with the radial growth factor $\kappa(r/R)$ included and without it, i.e., considering uniform growth but with different stiffnesses in the cambium region. The results of this analysis are shown qualitatively in Figure 4.8 and quantitatively in Figs. B.2, B.3 and B.4. For both uniform and radially modulated growth, we set the parameters defined in table 4.1 as our standard conditions. Note that the tissue expands overall faster under uniform growth as the radial growth factor is strictly $\kappa(r/R) < 1$. Therefore, to not skew the comparison, we do not consider the tissues after the same amount of simulation steps but rather once they reach the same area threshold. This means that uniform growth up until $t = 2.0$ corresponds to modulated growth with factor $\kappa(r/R)$ at $t = 4.16$. If not changing the stiffness of the cambium cells (Figure 4.8A.(ii), B.(ii)), we already know the results with the distinct stress pattern emerging. For uniform growth, however, like previously with the hexagonal meshes, as expected, we do not find any distinct pattern. This absence of patterning is more clearly visible also by the quantitative analysis of stress angles and magnitudes in Figs. B.3A.(ii) and B.4A.(ii). For increasing the stiffness in the cambium, however, to five times the reference, $\tilde{E} = 5 \cdot E_0$, also for uniform tissue growth we indeed do observe circumferential stress to emerge in the cambium (Figs. 4.8A.(iii) and B.3A.(iii)), resembling the result of modulating growth with the radial growth factor. If $\kappa(r/R)$ is used alongside an increased cambium stiffness (Figure 4.8B.(iii), the stress pattern does not change structurally but is slightly reinforced (cf. Figs. B.3B.(iii) and B.4B.(iii)). Another interesting situation appears if changing the cambium stiffness to only a quarter of the reference as shown in Figs. 4.8A.(i), B.(i). For the tissue grown with growth factor $\kappa(r/R)$, circumferential stress in the cambium for the larger stiffness values used is slightly suppressed. This effect results from increased cambial growth due to the locally reduced stiffness. Thus, the stress pattern is less prominent, which also shows in the angle distribution and stress magnitudes at this radius range (Figs. B.3B.(i) and B.4B.(i)).

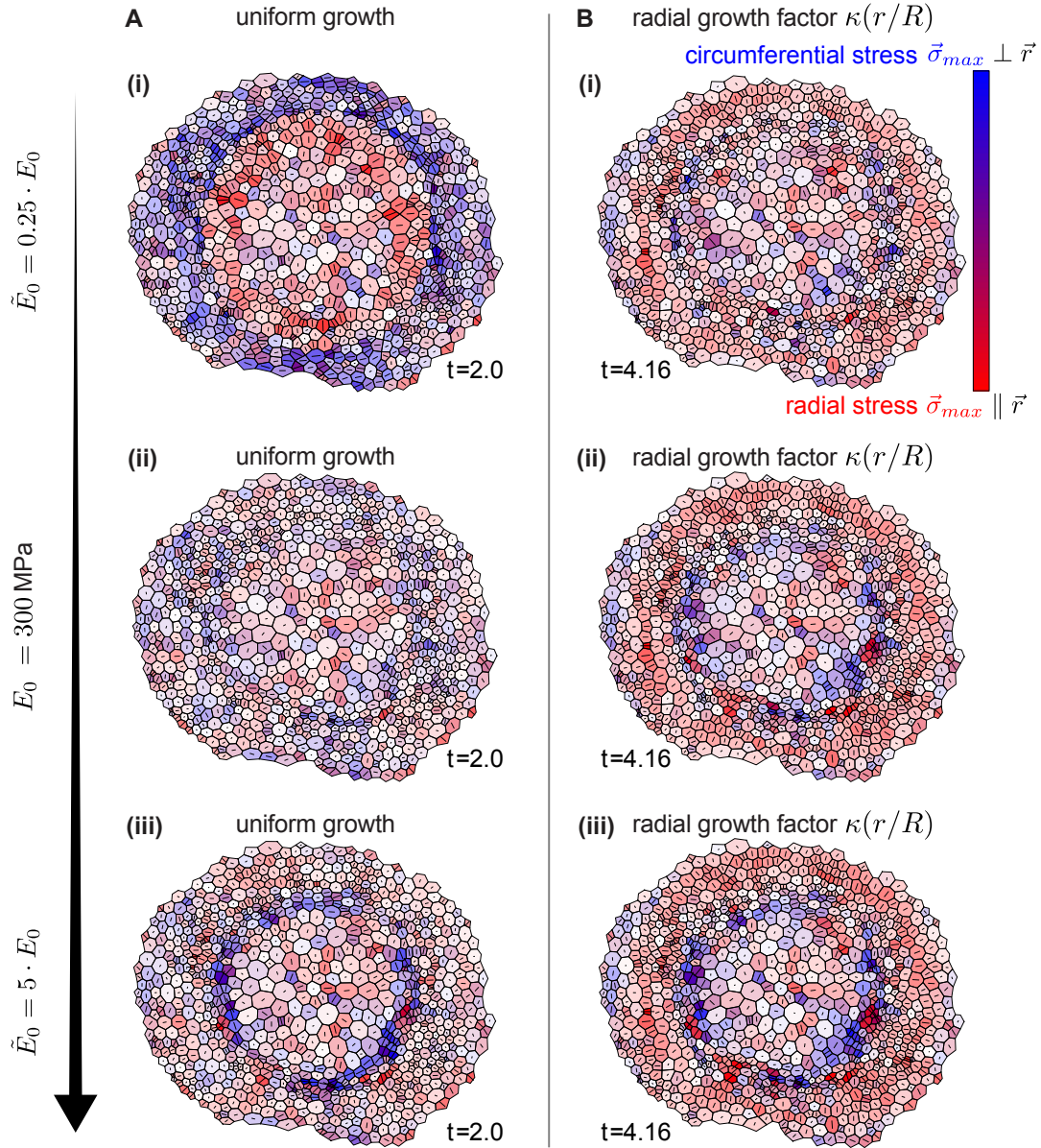


Figure 4.8: Differential growth can be introduced by both a direct modulation of cell growth or by changing the mechanical properties of cells in the tissue. Uniform growth patterns (A) and growth modulation by a radial growth factor (B) are compared for variations in the mechanical properties of the cambium cells in a radial range of 7.5%, i.e., $r/R = (r/R)_{min} \pm 0.075$ around the minimum of the center of the cambium region. Since the different growth patterns grow at different speeds, tissues are grown until they reach the same area threshold. Standard conditions of reference stiffness $E_0 = 300 \text{ MPa}$ are depicted in (A.(ii),B.(ii)). While for the radial growth factor, we may observe the concentric rings as a stress pattern, for uniform growth, no distinct pattern arises. Lowering the stiffness of cambium cells leads to high circumferential stress in the phloem (A.(i)) due to the now substantially increased radial growth of cambium, exerting forces onto the phloem. On the contrary, for radial growth (B.(i)), the circumferential stress in the cambium largely vanishes, showing a countering effect of decreased stiffness towards slower growth at this radial range (cf. Figure 4.3C). If cambium cell stiffness increases (A.(iii),B.(iii)), high circumferential stress arises in the cambium in both growth patterns, demonstrating the interchangeability of the growth factor $\kappa(r/R)$ and stiffness E_0 .

For uniform growth, though, we fascinatingly observe an inversion of the stress pattern compared to the pattern in panels B.(i) or A.(i). This inversion results from the cambium expanding faster than when using larger stiffness values, as the cambium keeps up with the expansion of the surrounding xylem and phloem tissue. As such, the phloem now represents the bottleneck of tissue expansion, and the expanding cambium continuously stretches the surrounding phloem tissue. This process is the inverse effect of slower-growing cambium, where it experiences circumferential stress by opposing the expansion of the xylem and phloem. In summary, these results show the potency of adjusting the mechanical parameters of cells, such as the stiffness.

4.3.3 Variations of turgor pressure

Next, we want to look into the effects of changing the turgor pressure of cells in the tissue. For this purpose, we again use the standard set of mechanical parameters described in table 4.1, but now we aim to explore changes by adjusting the turgor pressure of all cells in the tissue. The results of this endeavor are displayed in Figure 4.9 with additional quantitative details in Figure B.5. Here, we compare the area growth and stress magnitudes for different turgor pressure values at different simulation times. From Figure 4.9A, we can see that several combinations of simulation times and turgor pressures lead to essentially the same outcomes. For instance, growing a tissue for either 500 time steps (i.e., until $t = 5$) at turgor pressure $P = 0.5$ leads to very similar results as growing the same tissue for only half the time steps but with double the turgor pressure. Thus, we can deduce that turgor pressure is the main driver behind tissue growth in the real world and our simulations. Another interesting perspective we get from the direct correlation of the value for turgor and the stress magnitudes by computing the Frobenius norm of the stress tensors $\|\sigma\|_F$ of cells w.r.t. the relative radius r/R (Figure 4.9B-C). Here, we notice that the overall stress distribution in the tissue along the radius does not change, no matter the turgor pressure used. What does change, however, is the total values of the Frobenius norms as highlighted in Figure 4.9C. Thus, only the magnitude of stress changes but not the actual result or stress patterning, as can be seen quantitatively from Figure B.5, too. However, the similar results in the stress patterning do not mean that local turgor changes in one or more cells would not affect their surroundings. The important aspect is the occurrence of anisotropy between the mechanical properties of cells. A combination of the change of turgor and stiffness and its effect, we will pick up again and explore in Chapter 5 in the context of cell ablation, which is accompanied by a local drop of turgor pressure via destruction of individual cells or parts of the tissue.

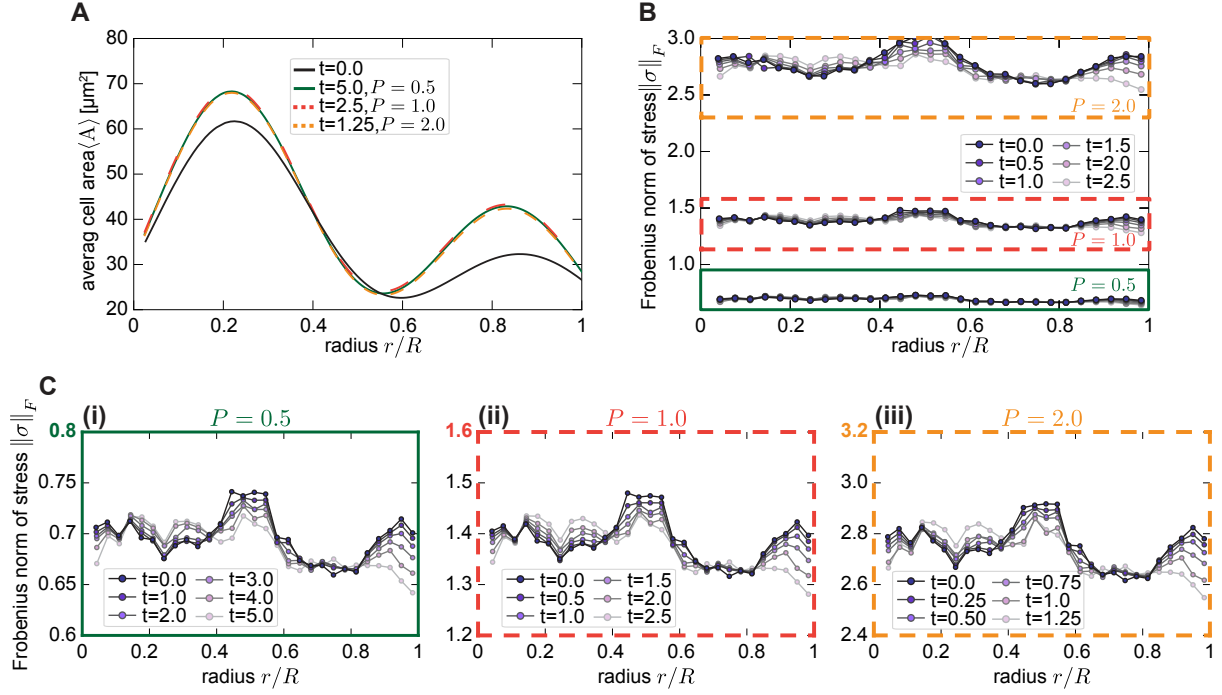


Figure 4.9: Changing turgor pressure only creates an offset of for total stress. (A) Comparison of the area distributions of cells of the tissue w.r.t. the relative radius r/R for different combinations of simulation time (step size $\Delta t = 0.01$) and turgor pressure P . The overlap of the curves illustrates their interchangeability. (B) Measure of total stress via Frobenius norm $\|\sigma\|_F$ w.r.t the relative radius r/R for turgor pressures of $P = 0.5$, $P = 1.0$ and $P = 2.0$ for the same simulated times, showing turgor to create an offset of total stress in the tissue. (C). (i)-(iii) Time evolution of the total stress via Frobenius norms for the different parameter combinations presented in (A). Note the different y-scales in panel (C).

4.3.4 Variations of Poisson's ratio

Poisson's ratio is the remaining mechanical parameter we intend to explore in this chapter. It is also an important parameter because it measures how a rigid body, like a cell, responds to deformation and, thus, the directional feedback to deformation. Essentially, this means that for a high Poisson ratio, the response to deformation is higher due to getting closer to the volume-preserving threshold of $\nu = 0.5$ ¹⁶⁰. Consequently, for a higher Poisson ratio, one would expect the tissue to be more constrained during its growth. Putting this hypothesis to the test, we perform again simulations of the radial growth of our experimental tissue, including the radial growth factor $\kappa(r/R)$ with standard growth conditions as described in table 4.1. Here, we explore the typically used Poisson ratio of $\nu = 0.3$ and a higher value of $\nu = 0.4$ for growth simulations up to $t = 5.0$. The results of this analysis are shown in Figs. 4.10 and B.6. Again, as with the turgor pressure analysis, the stress patterns (Figure B.6) do not significantly change between both parameters. What does change, however, is the increase

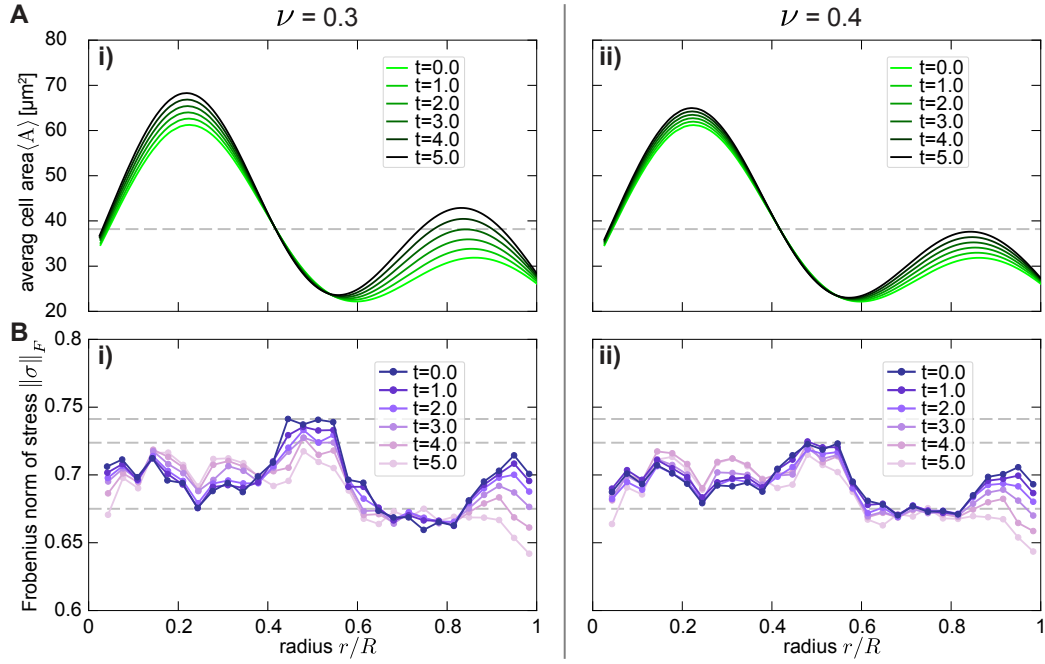


Figure 4.10: Increasing Poisson’s ratio slightly reduces total tissue stress. (A).(i)-(ii) Comparison of the average cell area w.r.t. the relative radius r/R for different Poisson ratios of $\nu = 0.3$ and $\nu = 0.4$. Increasing Poisson’s ratio to $\nu = 0.4$ results in slightly reduced tissue growth. Dashed lines indicate the threshold of identical areas at $t = 2.74$ and $t = 5.0$ for $\nu = 0.3$ and $\nu = 0.4$, respectively. (B).(i)-(ii) Comparison of the total tissue stress by the Frobenius norm of the stress tensor w.r.t. the relative radius r/R . Increasing Poisson’s ratio to $\nu = 0.4$ results in a slight decrease of overall stress due to slower growth but yields qualitatively the same distribution. Dashed lines are for better visual comparability.

in area and total stress, measured by the Frobenius norm of the stress tensor. For a lower Poisson ratio of $\nu = 0.3$, we observe both a higher increase in cell area (Figure 4.10A.(i)-(ii)) as well as a higher total stress in the cambium (Figure 4.10B.(i)-(ii)). The latter is, however, entirely attributable to the accelerated growth in comparison to the results for $\nu = 0.4$. We find that this is because the tissues grow at different paces. In fact, the results are almost identical when comparing the different values for ν not after the same number of simulation steps Δt but after tissue growth has reached a certain area threshold. Similarly to our analysis of changing the stiffnesses, changing Poisson’s ratio leads to combinations of $t = 2.74, \nu = 0.3$ and $t = 5.0, \nu = 0.4$ correspond to the same area threshold (dashed lines in Figure 4.10A). At these steps, the results do not differ significantly. Ultimately, changing Poisson’s ratio mostly seems to lead to a slight reduction of growth when simulating for the same number of simulation steps. In turn, increasing Poisson’s ratio hints towards a higher degree of feedback of cells under deformation, restricting growth to some extent. Considering Hooke’s law, this also becomes clear when considering that the Lamé parameters can also be expressed by $\mu =$

$\frac{E\nu}{(1+\nu)(1-2\nu)}$ and $\lambda = \frac{E}{2(1+\nu)}$. Increasing Poisson's ratio from $\nu = 0.3$ to $\nu = 0.4$ causes λ to increase faster than μ , giving rise to a higher contribution of the volumetric stress term $\lambda\epsilon_{kk}$ in Hooke's law (Eqn. 3.27), ultimately penalizing deformations via the elastic energy in the Hamiltonian (Eqn. 3.40). Still, this difference is arguably less impactful than those from the previously discussed parameters.

4.3.5 Interim summary and conclusion

In this chapter, we successfully established a procedure to generate simulation meshes from artificially created point patterns via Delaunay triangulation and from segmentations of microscope images. For both cases, we derived a radial growth factor $\kappa(r/R)$, capturing the growth of tissues based on the change in area from experimental data collected in earlier studies⁴⁴. We demonstrated that uniform growth does not result in stress patterning in the tissue. However, with the modulation of growth, we found distinct circumferential stress patterns within our tissues, which are also robust against different geometries and topologies for modulations of the tissue dynamics with the radial growth factor. Furthermore, we analyzed changing the mechanical properties of cells in the tissue, namely Young's modulus, turgor pressure, and Poisson's ratio. From there, we saw that altering the stiffness regarding the simulation results is mainly equivalent to modulating growth by a radial growth factor. Note the significance of this finding, showing that either the rate of cellular growth with uniform stiffness or uniform growth with modulated stiffness produces a highly similar result. In the latter case, we only took a very makeshift approach of modulating the stiffness like a step function, unlike the approach we took for the radial growth factor. On the other hand, it is also important to point out that such a scenario of the highest stiffness occurring in the cambium is not biologically reasonable, as xylem tissue should be much stiffer in reality as it provides the backbone of the growing plant. Still, it shows the complexity behind plant tissue development as, in turn, the difference in area growth that is observed in experiments needs to originate from another source. One major contribution to this is cell divisions, the dynamics of which we discuss in Chapter 5. Furthermore, we saw that turgor pressure, if changed globally for all cells, only offsets the overall tissue stress and energy by contributing to the Hamiltonian. In contrast, the actual stress patterning remains the same.

Regarding Poisson's ratio, increasing this parameter showed an overall reduction in growth due to increasing constraints towards deformation and, thus, growth itself. This observation also hints at a degree of growth-mediated mechanical feedback. While not having such mechanisms considered any further in this chapter, we will partially pick up the topic of

mechanical feedback and its effect again in Chapter 5. In summary, in this chapter, we could get diverse insights into the behavior of our growth simulation in dependence on simulation meshes and mechanical parameters and constraints. One could find all sorts of combinations of mechanical parameters, adapting them for individual cells or, for instance, radially along the tissue. However, due to the prevalent absence of experimental evidence, we stick to modulating only the growth rate of cells as a reasonable input for this work, as we may base it on the currently available experimental analyses. The precise origin of the differential growth that this data suggests remains unclear, though. Future experimental measurements are required to decipher the underlying mechanisms for these experimental results.

In the following chapter, we will now turn our attention towards simulations of cell divisions in the context of radial growth of the hypocotyl of *Arabidopsis thaliana* to uncover the emergence of cell files and to relate the entirety of cell division in the cambium to mechanical forces alongside further analyses of the tissue dynamics under various mechanical perturbations.

5

Publication: Radial growth in the hypocotyl of *Arabidopsis thaliana*

The following chapter is based entirely, including all text and figures, on the publication:

Mechanical forces instruct division plane orientation of cambium stem cells during radial growth in *Arabidopsis thaliana*¹³⁰

by Mathias Höfler^a, Xiaomin Liu^b, Thomas Greb^b and Karen Alim^a

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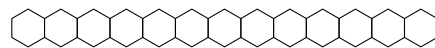
<https://doi.org/10.1016/j.cub.2024.10.046>

To the conceptualization and methodology of this publication, all authors contributed, but especially Karen Alim and Thomas Greb. Simulations and evaluations were carried out by Mathias Höfler. Experimental data was collected and experimental analyses were carried out by Xiaomin Liu. The manuscript was written by Mathias Höfler with the help of Xiaomin Liu, Karen Alim, and Thomas Greb. Further individual contributions in detail are described in Author contributions section of this chapter.

Apart from the additional summary below, all content is identical to the published version, except for minor spelling changes.

Summary:

This chapter explores the radial growth of the hypocotyl of Arabidopsis thaliana. We integrate insights from plant physiology and computational modeling of plant tissue to investigate the connection between mechanical forces and the orientation of cell divisions. These divisions occur in the vascular cambium, the secondary meristematic tissue mainly responsible for radial growth. Our study combines an anatomical analysis of experimental data with numerical simulations of tissue growth and cell division, using a mechanical model and growth modulation based on experimental evidence. The central question addressed in this chapter is how cell division is organized within the cambium and whether mechanical stress is a key factor in determining the division plane orientation. Our simulations align with the experimental observations, which we demonstrate by investigating both the in silico and in planta response to mechanical perturbations of the system. Notably, only cell divisions based on mechanical stress produce the experimentally observed pattern in division plane placement, leading to the emergence of cell files in the cambium. Thus, we find cell files to be a consequence of the prevalent stress pattern resulting from the differential growth of the cross-section. This stress pattern was also robust against local but not global perturbations, as confirmed by our experimental analysis. Altogether, our results highlight the central role of mechanical forces as key regulators of precise and robust division wall placement and, thus, the radial growth and tissue organization in the hypocotyl of Arabidopsis.

**5.1 Abstract**

Robust regulation of cell division is central for the formation of complex multi-cellular organisms and a hallmark of stem cell activity. In plants, due to the absence of cell migration, correct placement of newly produced cell walls during cell division is of eminent importance for generating functional tissues and organs. In particular, during radial growth of plant shoots and roots, precise regulation and organization of cell divisions in the cambium are essential to produce adjacent xylem and phloem tissues in a strictly bidirectional manner. While several inter-cellular signaling cascades have been identified to instruct tissue organization during radial growth, a role of mechanical forces in guiding cambium stem cell activity has been frequently proposed but, so far, not been functionally investigated on the cellular level. Here, we coupled anatomical analyses with a cell-based vertex model to analyze the role of mechanical stress in radial plant growth at the cell and tissue scale. Simulations based on segmented cellular outlines of radially growing *Arabidopsis* hypocotyls revealed a

distinct stress pattern with circumferential stresses in cambium stem cells which coincided with the orientation of cortical microtubules. Integrating stress patterns as a cue instructing cell division orientation was sufficient for the emergence of typical cambium-derived cell files and agreed with experimental results for stress-related tissue organization in confining mechanical environments. Our work thus underlines the significance of mechanical forces in tissue organization through self-emerging stress patterns during the growth of plant organs.

5.2 Introduction

Cambium-driven radial growth is a fundamental growth process in many plants increasing the circumference of their shoots and roots by the production of vascular tissues. In the form of wood (xylem), vascular tissue production contributes by a large proportion to the world's total production of biomass²¹⁵. Radial growth generally is driven by cambium stem cells which reside in a cellular environment hosting very rigid tissues while generating xylem and phloem cells in a bi-directional manner⁴² (Figure 5.1A). Xylem cells are usually produced inwards and ensure long-distance water transport as well as mechanical stability by their well-developed secondary cell walls. Phloem cells are produced outwards and play an important role in transporting sugars and other organic molecules^{34,42,58}. Cambium-derived cells often display very organized alignments in radial orientation (Figure 5.1A). While cambium cells occasionally divide radially (anticlinal) in order to increase the girth of the tissue²¹⁶, this overall organization is based on a strong preference for tangential (periclinal) divisions of cambium stem cells that contribute to the radial expansion of the tissue and for which mechanical forces have been proposed to be instructive¹⁹. In lack of a clear explanation for the orientation of cambium stem cell divisions, concentration gradients of peptides secreted by phloem cells have also been suggested to serve as instructive signals²¹⁷.

The hypocotyl of *Arabidopsis thaliana* is the organ connecting the shoot and the root system and serves as a versatile model for investigating radial plant growth, including morphometric analyses and *in silico* simulations of cambium-based organ expansion^{43,44,125,218,219}. These simulations have partly assumed inter-tissue forces generated by developing xylem cells instructing cambium stem cells' division orientation⁴³. However, the pattern of mechanical forces in radially growing plant organs have not been revealed yet, let alone their impact on dividing cambium stem cells in a natural context. Various division rules were proposed for plant cells in the past. The most prominent of those rules is that "the cell plate, at the time of its formation, adopts the geometry that a soap film would take under the same conditions" (Errera's rule)⁷⁴, deriving from the observation that cells often behave like surface-minimizing

soap bubbles. Therefore, the rule emerged that divisions take place along the plane that minimizes the surface area of the cell, leading to daughter cells of same size^{74–79}. For the case of the *Arabidopsis* embryo, if extending Errera's rule to three dimensions and comparing the area difference between possible division planes very precisely, division along the plane of minimal surface area has been shown to apply even if the area difference between different division planes is minor^{220,221}. Errera's rule was furthermore revised suggesting the shortest axis as the most likely division plane⁷⁸. Still, cells also often deviate from this 'shortest axis' rule and purely geometrical rules usually do not accurately capture the dynamics of division plate placement^{19,97,220,222}. Consequently, additional mechanisms have been brought into discussion. In the case of the shoot apex, Louveaux et al.¹⁷⁶ provided a reconciliation of Errera's rule and mechanical cues, where the shortest plane matches the largest tension. It is important to point out that established cell division models often consider surface cells of shoots or roots which are typically under tension, while in this study we touch the comparably little studied field of internal tissues undergoing radial growth²²³. An important aspect of hypocotyl development is that it occurs in phases^{116,117} (Figure 5.1B). Hypocotyls experience a first phase of longitudinal elongation, followed by a second phase of radial growth, considered here, when longitudinal growth has ceased. In this radial growth phase, neither cambium stem cells nor their derivatives divide anymore transversely^{82–84}. Due to the absence of transverse divisions, Errera's rule does not capture the division pattern in the elongated cambium stem cells. While auxin signaling was shown to be involved in radial growth and promote deviations from Errera's rule^{52,224}, there is, however, strong evidence that plant cells respond to mechanical stresses on a local or even tissue level, possibly impacting cell divisions^{19,77,78,80,81,97,176,220,222,225–234}.

Plant cell shapes depend largely on internal turgor pressure and the mechanical properties of surrounding cell walls¹⁸¹. Mechanical forces not only emerge from individual cellular properties though, but especially from the tissue- and organ-wide arrangement and expansion of cells due to the tight connection of cells via their walls^{25,73,78,182,183,223,235}. In consequence, estimation of the overall stresses arising in an internal tissue like the cambium requires both, stresses emerging from the individual cell shape, as well as stresses arising within the tissue from the neighboring cells and the forces they exert^{25,35,182,183}. Shape-derived stress can be estimated by approximating cells as pressurized cylinders (Figure 5.1B, left) where transverse stress is a priori twice as large as longitudinal stress. However, tissue-derived stress contributions have to be considered as well and were reported to exceed shape-derived stresses^{25,70,182,183}. Various cell structures align with the direction of maximum tensile stress, most notably cortical microtubules which orient in parallel to the maximal tensile stress

direction^{23,60,79,176,236–238}. That implies that tension caused by mechanical changes in neighboring cells, for example through ablation or other mechanical perturbations, manifests by a reorientation of CMTs¹⁸. As a consequence, the orientation of cell division planes may thereby be affected^{79,176}. During the initial elongation phase of the *Arabidopsis* hypocotyl, the hypocotyl increases in length by at least six-fold without significant radial growth^{31,122}. From this strong growth anisotropy, growth-derived stress arises, that was shown to exceed shape-derived stresses in the hypocotyl^{25,70} (Figure 5.1B, middle). In particular, measurements of Poisson's ratio of inner tissues in hypocotyls elucidated that tissue expansion leads to longitudinal stress exceeding transverse stress^{25,182,183}. It is, thus, likely that even after the initial elongation phase of the hypocotyl has ceased, residual tissue-derived stresses from the elongation growth phase comprise mechanical cues during the radial growth phase. These stresses may combine with forces exerted to the cambium from its surrounding tissues during radial growth such that, overall, tissue-derived stress dominate over shape-derived stress. We therefore hypothesize that the major stress direction aligns with the longitudinal direction of the hypocotyl, i.e. the plane of maximal stress is perpendicular to the plane of minimal area. While, in consequence, the division plane and mechanical stress seem highly connected, the strictness of the divisions during radial growth in either anticlinal or periclinal direction is not answered as both are in longitudinal direction of the hypocotyl. Instead, explaining the division orientation requires considering morphogenesis in direction of the tissue expansion which is the transverse direction as revealed by two-dimensional cross-sections of the hypocotyl. There, unlike in the longitudinal direction, both the tissue geometry and mechanics are directly affected from radial growth.

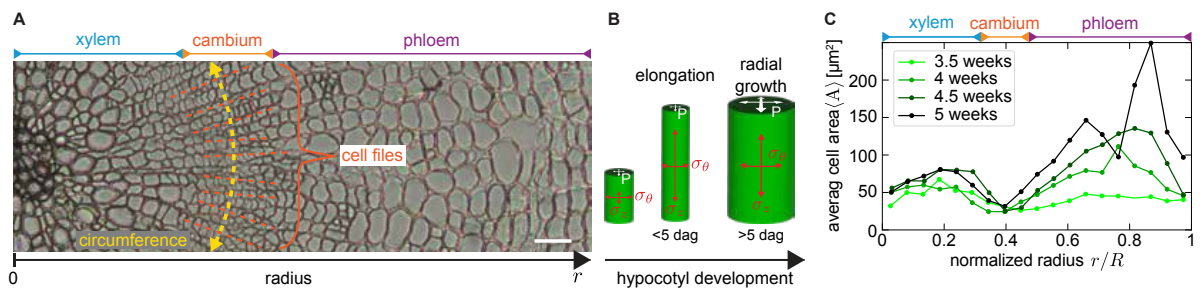
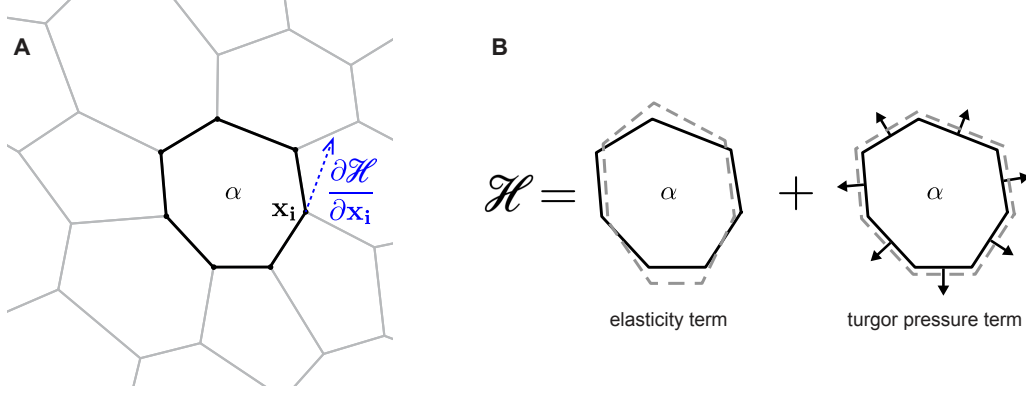


Figure 5.1: Cambium tissue is organized into cell-files and cell areas in the hypocotyl show a bi-modal distribution. (A) cross-section of an *Arabidopsis* hypocotyl. Cambium-derived cells are arranged in cell files (orange dashed lines) showing a high level of organization, compared to xylem and phloem tissue. Scalebar 10 μm. (B) Sketch of hypocotyl development timeline in days after germination (dag). After rapid elongation, longitudinal growth ceases and transitions to the here considered radial expansion (white arrows). Maximum stress experienced by the internal tissue is depicted by red arrows. (C) Binned average cell area distribution (bin size 0.05) as a function of the normalized tissue radius r/R with respect to the tissue center shows a bi-modal distribution at all ages. Underlying data from⁴⁴.

Box 1. Cell-based vertex model description



Vertex model theory:

The tissue is described by a cell-based vertex model in two dimensions. Cells are represented as a graph of connected vertices, defining encircled polygons (A). Polygons have an area $A_{0,\alpha}$ and are numbered by $\alpha = 1 \dots N$. The cell mechanical properties are represented by the energy function $\mathcal{H}$, consisting of a term for the elastic energy of cells and pressure within cells (B)

$$\mathcal{H} = \frac{1}{2} \sum_{\alpha=1}^n A_{0,\alpha} (2\mu \text{Tr}(\varepsilon^2) + \lambda [\text{Tr}(\varepsilon)]^2) - \sum_{\alpha=1}^n P A_{0,\alpha},$$

with strain ε , Lamé constants μ and λ and turgor pressure P . We follow a quasi-static approach in order to find stable tissue configurations that satisfy the force balance such that the sum of forces F_i at each vertex position x_i vanish, thereby locally minimizing the energy $\mathcal{H}(x_i)$. The first sum of the energy function represents the elastic energy of the tissue taking into account the Lamé parameters μ and λ and the strain ε acting on each cell α , which is derived from the mismatch between the observed cell shape and the cell shape at minimized energy. The second sum is a volume term, assuming a constant cell height normalized to one, and represents the turgor pressure of the cells. Related to STAR★Methods.

In this work we investigated if mechanical stresses, arising during radial growth, are instructive in orchestrating coordinated tissue growth and arrangement. To this end we started with a quantitative analysis of radial growth of the *Arabidopsis* hypocotyl. By performing two-dimensional simulations of cross-sections starting from segmented cell outlines within a vertex model, we found a distinct pattern of circumferential stress in cambium stem cells and radial stress in the xylem and phloem. Our simulations revealed that stress-oriented cell divisions explain the emergence of ordered tissue structures during radial growth and, thus, that organ-wide stress patterns are prominent candidates for decisive positional cues during cambium-dependent tissue production. This conclusion was supported by disrupting tissue stress patterns through applying exogenous mechanical stress which led to the same disorganized tissues pattern in both simulations and experiments. Based on these observations, we propose that cell divisions oriented along mechanical stress vectors are central for organized radial growth of plant organs.

5.3 Results

Cell-based vertex model captures radial hypocotyl growth

To assess the disparity in tissue type growth for deducing organ-wide mechanical stress patterns, we took advantage of previously published morphometric analysis of hypocotyl cross-sections⁴⁴. Note, that hypocotyl samples considered here are at comparable longitudinal position along the hypocotyl as in⁴⁴ and assumed to be at the same developmental stages. As is shown in Figure 5.1C, we found both xylem and phloem cells to be much larger in surface area compared to cambium cells. Thus, mapping out the average cell area $\langle A \rangle$ versus the normalized radius r/R from the tissue center, with R being the total radius of the tissue, we found the area distribution to be bimodal. It was noticeable that the average xylem cell area increased only very slowly over time, while phloem cells grew much more within the same time interval. Cambium stem cells, on the other hand, kept on average the same size throughout tissue development. To initialize mechano-simulations by a cell-based vertex model, we first segmented an image of a two-dimensional cross-section (Figure 5.2A) from an *Arabidopsis* hypocotyl, produced for this study, using the PlantSeg/MorphographX software packages^{91,211} generating a realistic tissue mesh. As a compromise between accuracy and computational practicability, from this segmentation we generated a Voronoi mesh (Figure 5.2B), using the centroid for each cell in the segmentation. This resulted in a simplified tissue structure that retained, up to minor deviations, the quantitative characteristics of the original tissue, like the average cell area as a function of the radius (Addendum Figure 5.6A) and the cell anisotropy

(Addendum Figure 5.6C,D), i.e. the orientation of the short axis of each cell. Note that we chose this coarse-graining only retaining few quantitative properties from the segmentation on purpose, in order to uncover any emerging behaviour in stress and division patterns, as well as the cell file structure in the cambium independently of the initiation of the tissue geometry. In our model, we represented each cell by a polygon, defined by a series of vertices, connected by straight lines, i.e. edges (Box 1A).

As contributions to the tissue energy we took into account the turgor pressure and the elastic energy of each cell (Box 1B). The elastic energy comprised the strain of each cell, which was proportional to the mismatch between the observed, i.e. deformed, shape of the cell M_c and the undeformed cell shape M_0 (Figure 5.2C and Eq.5.2). The undeformed cell shape hereby evolved in time by applying exponential growth to the system (Eq. 5.4). In addition, mechanical feedback accounted for the impact of CMTs on cell growth direction by promoting anisotropic cell growth perpendicular to the direction of maximum stress⁶⁷. We followed a quasi-static approach such that in each simulation step, an optimization was performed in order to find a local minimum of the tissue energy, $\mathcal{H}$ (Eq.5.5). This corresponds to a stable configuration in which the forces on each vertex were balanced, resulting in a vanishing net force. For cell divisions, we defined division planes through the center of the dividing cell where the direction of division was determined depending on the division rule applied. The strain ε acting on the mother cell before the division was conserved such that both daughter cells combined experienced the same strain as the mother cell had been subject to before the division. This was achieved by matching the undeformed shape matrices of daughter cells to the undeformed shape matrix of the mother cell (Eq.5.10). In this way, daughter cells both inherited proportionally the amount of strain, depending on the ratio of their surface areas (Figure 5.2E). It was only after the division event, when a new local energy minimum was sought, that the tissue adapted to the newly introduced degrees of freedom and the polygonal network adapted accordingly to the mechanical forces.

Because our simulations intended to study the role of mechanical stresses in driving cell patterns in cambium-derived tissues, we focused on the *in silico* emergence of tissue structures during the early growth phase of the hypocotyl. A major challenge in this undertaking was the lack of many desired input parameters for the simulations such as the mechanical properties of different cell or tissue types and their dynamics during cell division and tissue development. We therefore used available data⁴⁴ in order to indirectly extract this information. All cells were mechanically treated equally in the simulations and we did not distinguish between different types. Differences in mechanical properties of the cells were taken into account

indirectly by applying a radius-dependent growth factor $\kappa(r/R)$ (Figure 5.2D) where r was the Euclidean distance to the tissue centroid and R the radius of the tissue. This captured the observed evolution of the radial distribution of cell areas⁴⁴. The cell area distribution in both younger and older hypocotyls was considered bimodal²³⁹ (Figure 5.1C, Addendum Figures 5.6A, 5.6G) and differed in the average cell area of xylem and phloem tissue over time. Morphometric data⁴⁴ moreover suggested a massive cell growth in phloem tissue, implying the growth rate in the phloem to be larger than in the xylem. For this reason, the xylem makes up a comparably larger proportion in younger hypocotyls while, during aging, the phloem area massively increases. Therefore we used a growth function $\kappa(r/R)$ which replicated this transition. For determining this function, we started to fit the sum of two Gaussians to the area data of our Voronoi mesh, obtained from segmentation. These took the form $f(r) = a_1 \exp(-(\tilde{r} - b_1)^2/c_1^2) + a_2 \exp(-(\tilde{r} - b_2)^2/c_2^2)$, where $\tilde{r} = r/R$ was the normalized

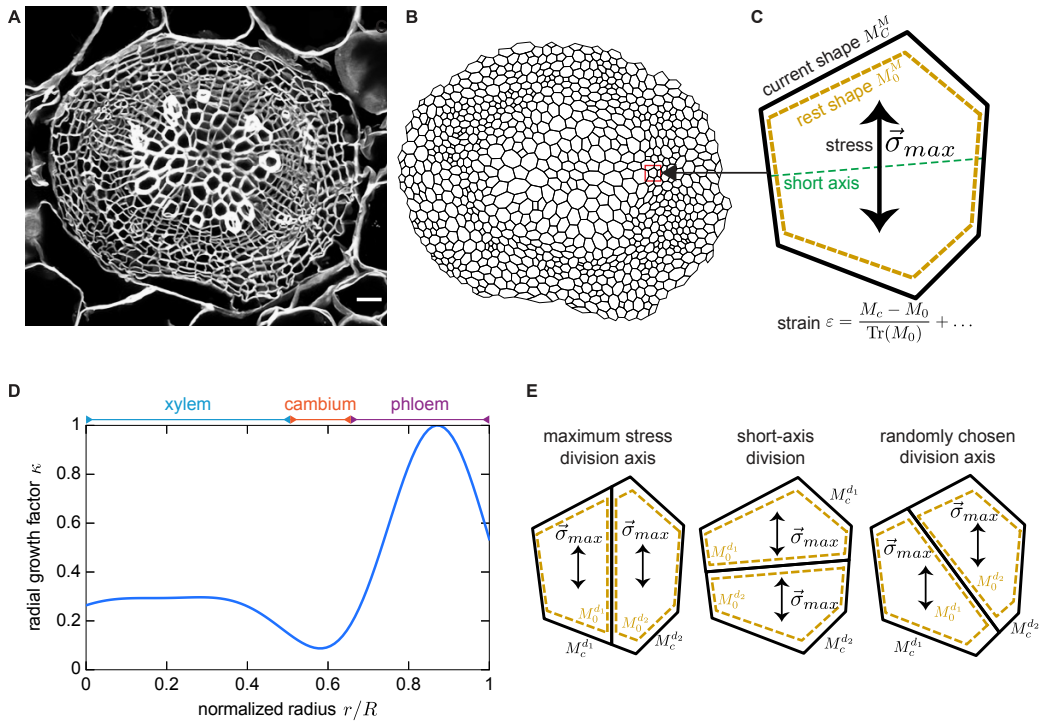


Figure 5.2: *In silico* recreation of segmented tissue and observed cell growth rate form basis for tissue level simulations, including cell divisions (A) cross-section of a two-week old *A. thaliana* hypocotyl that served as simulation template. Scalebar 15 μm (B) Voronoi tessellation as simulation input, obtained from segmentation of A. (C) Description of individual cell geometries by means of shape matrices. (D) Radial growth function used to modulate individual cell growth as a function of radius. (E) Schematic of maximum stress, short-axis and random division rules for division plane placement, investigated in simulations. Details about the mesh properties and the radial growth function in Addendum Figure 5.6. Details on the cell division procedure in the STAR*Methods.

radius. We combined the fit of the Voronoi mesh at two weeks of age (Addendum Figure 5.6A) with the experimental data at later stages (Figure 5.1C) to compute a growth rate that evolved the area distribution of the young sample's Voronoi mesh such that it eventually matched the experimental data after growth. Since we assumed a Gaussian distribution we could determine the slopes of amplitudes A_i and means μ_i for each distribution of the experimental data (Addendum Figure 5.6H,I) over time and considered this the rate of change of the respective quantity within half a week of growth between 3.5 and 5 weeks old samples. This slope was added to the parameters that described the Gaussian fit of the area distribution of the Voronoi mesh (Addendum Figure 5.6J), therefore corresponding to the theoretically expected area distribution at a sample age of 2.5 weeks.

Additionally we adjusted the fit's variance such that the x-position of the maxima and the local minimum of the original distribution of the Voronoi mesh were retained. This was necessary as increasing the amplitudes would have otherwise artificially shift the extreme values due to the summation of both Gaussians. Taking the difference of this distribution, which would be corresponding to a 2.5 weeks old sample, and the experimental distribution at the age of 2 weeks, we obtained a radial growth function $\kappa(r/R)$ (Figure 5.2D and Addendum Figure 5.6J) which we used to modulate individual cell growth, depending on the radial position of the cell center. Note that for large radii the growth rate declines again recreating the impact of the epidermal cell layers confining growth.

Circular stress-patterns emerge from radially varying growth rates

Employing our model, we first looked into the mechanical stress patterns emerging dynamically within the tissue without cell divisions. The experimental observation of a bi-modal distribution of cell areas at all hypocotyl stages indicated xylem and especially phloem cells to be expanding faster than cambium cells. To implement the larger expansion rates, we increased for the simulations' initial condition the undeformed shape matrices M_0 for both xylem and phloem isotropically by 5% and 20%, respectively. Apart from this increase, we did not impose further initial conditions or any stress patterns. Instead, the initial stresses were determined from the energy optimization during the first simulation step. To quantify the emerging stresses for the subsequent analyses, we always used the tissue center as a reference point. Moreover, we averaged over 100 independent runs where individual cell growth underlied multiplicative Gaussian noise beyond the radial growth function $\kappa(r/R)$. This ensured stochastic growth of each individual cell. The stress angle (Figure 5.3A), i.e. the angle of the direction of maximum stress, we defined with respect to the periclinal direction of the tissue, therefore the normal to the radius vector. For each individual cell, we computed

the stress angle over the course of the simulation. In detail, we started by growing the tissue, obtained from the Voronoi tessellation of the segmented data, with a time step of $\Delta t = 0.01$. We then applied our radius dependent growth factor $\kappa(r/R)$ in every simulation step and continuously monitored both the average stress angle $\langle \theta(\vec{\sigma}_{max}) \rangle$ and the area distribution $\langle A \rangle$ as a function of the normalized radius r/R (Addendum Figure 5.6E,F). During growth of the initial mesh we observed the appearance of a distinct stress pattern, establishing already after a few simulation steps and resulting in clear concentric domains (Figure 5.3B). Our quantitative simulation analysis (Addendum Figure 5.6E) showed that already shortly after the beginning of the simulation, at $t = 1.0$, there was a distinct difference between the average stress angles $\langle \theta(\vec{\sigma}_{max}) \rangle$ along the hypocotyl radius.

At small radii up to about $r/R \approx 0.6$ stresses were largely oriented in periclinal direction whereas for larger radii, there was a clear transition towards the anticlinal direction. This pattern became more prominent over time (Addendum Figure 5.6E), also indicated by more pronounced transitions between periclinal and anticlinal stress along the radius of the tissue. For very small radii $r/R < 0.2$, due to the very small number of cells, we could not finally conclude the stress orientation, however the data suggested a rather radial orientation as well. Following our differential growth function, xylem and phloem cells grew both faster than cambium cells (Addendum Figure 5.6F), with phloem cells growing much faster than xylem cells, as desired from considering the experimental data (Figure 5.1C). At large radii, the growth rate declines again (Figure 5.2D), reflecting the tissue's confinement by the epidermal layer, visible by circumferential stress in peripheral cells (Figure 5.3B, peripheral cells on the left-hand side of the tissue). This effect we found to be more prominent if the growth rate at the peripheral cell layer was further reduced in order to probe the impact of mechanical changes in the epidermis (Addendum Figure 5.7A-D). Apart from the increase in circumferential stress at the periphery though, the stress pattern further inside the tissue remained virtually unchanged. Based on these observations we concluded that applying our growth function $\kappa(r/R)$ on the initial conditions, derived from experimental data, circumferential stress in the cambium and radial stress in the xylem and phloem emerged. This stress pattern is also robust against the choice of coarse-graining of the segmented cell shapes (Figure 5.2B, Addendum Figure 5.6L-N) and exact strength of mechanical feedback η (Addendum Figure 5.7E-J).

To challenge the relevance of the observed stress pattern, we next compared the effect of *in silico* removal of the xylem tissue in the simulation with the removal of the xylem tissue *in planta*. *In silico*, we simulated the cell ablation by setting the stiffness of cells to zero and reduced the turgor pressure to a residual ten percent²³. We focused on the xylem as from the

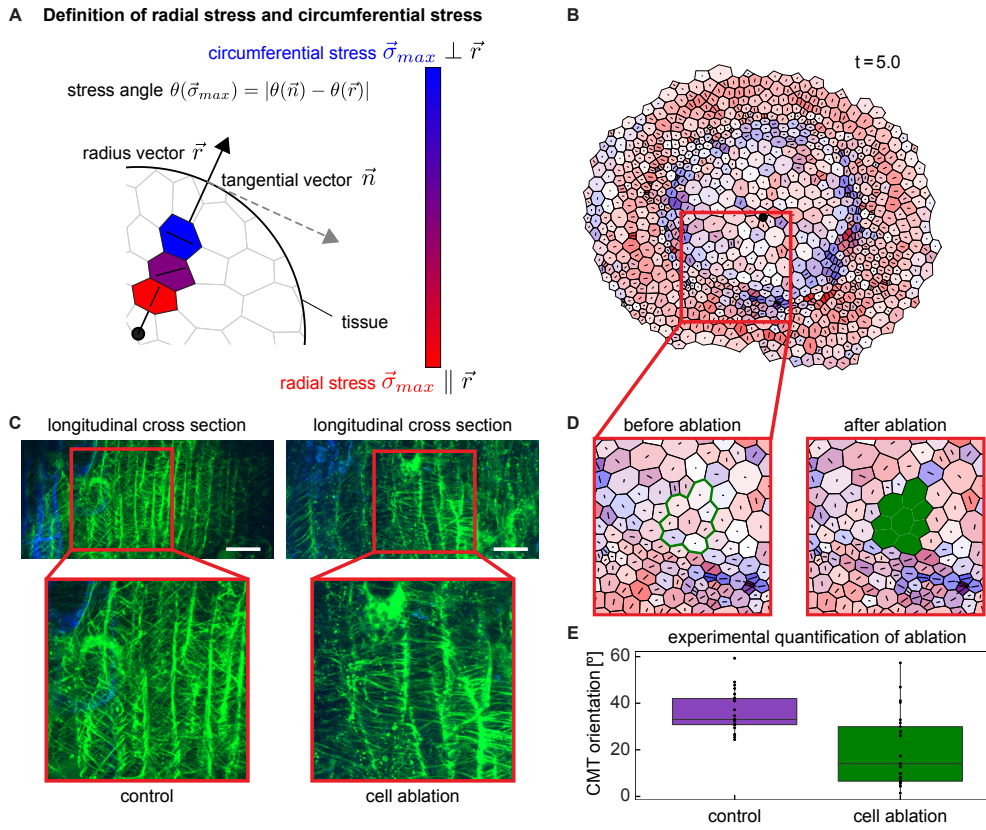


Figure 5.3: Simulations show a distinct stress pattern with circumferential stresses in the cambium and radial stresses in the xylem and phloem that respond also to mechanical perturbations. (A) Definition of the radial stress and circumferential stress, as well as the stress angle $\theta(\vec{\sigma}_{max})$. Measures are always with respect to the tissue center. The color bar captures the variation in stress between the extreme cases of the vector of maximum stress $\vec{\sigma}_{max}$ having either only a component in radial ($\vec{r}$) or circumferential ($\vec{n}$) direction. (B) Distinct circumferential stress pattern after growing the initial Voronoi mesh (Figure 5.2B) at $t = 5$ (500 iterations). Tissue center marked by black dot. (C) Longitudinal cross-sections of the hypocotyl with lignin in xylem vessels visualized by auto-fluorescence (blue) and CMTs visualized by *ypWOX4::mVenus-MBD*-derived signals (green). Scalebar 25 μm . In the control (left) microtubules are spirally winded around cells (see Video S1). After xylem removal (right), they are horizontally oriented, indicating a mechanical reinforcement towards the cavities. 38-day-old seedlings were used with three biological replicates included. (D) *In silico* ablation of xylem tissue of highlighted area (left). After ablation (right), both xylem and cambium cells surrounding the ablation site, are subject to higher stress overall and stresses re-orient parallel to the area of ablation. (E) Quantitative experimental analysis of CMT orientation angle w.r.t. horizontal axis of control, and after ablation. The angles in 25 individual cells were measured for the control and cell ablation treatment, respectively. Details about the growth and stress pattern in Addendum Figures 5.6E,F and 5.7.

growth differentials (Figure 5.1C) we saw that the phloem grows much faster than the xylem, therefore we intended to verify whether the xylem is able to impose a considerable growth pressure on cambium stem cells, even with its slower growth rate. Doing so, we observed an overall significant increase of stress around the ablation site (Figure 5.3D, right) even in cells at a few cell layer distance from the direct neighbors of the ablation site. There, stresses of

surrounding cells reoriented around the wound such that the maximum stress was parallel to the ablated tissue surface. Removal of xylem tissue also led to increased circumferential stress in the cambium (Figure 5.3D, right). To see whether our simulations faithfully predicted stress dynamics *in planta*, we experimentally visualized CMTs in cambium cells in longitudinal cross-sections of the hypocotyl. To this end, we used a line expressing the fluorescent mVENUS-MICROTUBULE BINDING DOMAIN (mVenus-MBD)²⁴⁰ fusion protein under the control of the cambium-specific *WUSCHEL RELATED HOMEODOMAIN 4* (*WOX4*) promoter⁴². After manual removal of the xylem tissue and a one-day recovery phase, we analyzed CMT organization in cambium stem cells by live-cell imaging. Compared to non-disturbed controls, where CMTs showed a prominent spiral twisting (Figure 5.3C,E, left; Video S1), CMTs in ablated samples were arranged more horizontally (Figure 5.3C,E, right). These observations let us conclude that, as predicted *in silico*, the xylem tissue applies pressure to cambium stem cells preventing their radial stretching. In our simulations we further considered ablations in multiple sites of the tissue (Addendum Figure 5.7K,L), including the phloem, which always resulted in increased stress. The biggest impact we found to happen if cambium tissue itself was ablated, leading to the highest stress response in the surrounding tissue, indicating the large forces that both the xylem and the phloem exert on the cambium as it also would be expected experimentally given the experimentally observed growth differentials (Figure 5.1C). We also concluded that our simulations, although abstracting the hypocotyl tissue as a two-dimensional cell mesh, correctly predicted enhanced circumferential stress specifically in cambium stem cells if ablating xylem or phloem tissue. Note that the oblique or spiral alignment of CMTs in the control is an effect of cessation of growth in the hypocotyl cells studied. CMT orientation in the hypocotyl is quite complex and drastically depends on the developmental stage of the tissue^{241,242}. During the rapid elongation of the hypocotyl, large axial forces are transmitted from the internal tissue to the epidermis which drive the elongation and transverse orientation of CMTs in inner tissue layers⁶⁰, associated with stress-relaxation of the yielding cell walls²⁵. This CMT order is however lost when longitudinal growth ceases, which is when oblique or longitudinal CMT arrays beneath the peripheral cell wall are found^{25,70,241,242} (see Video S1). Thus, mechanical stress especially at this stage represents a competition between shape-derived and tissue-derived stress, explaining the horizontal alignment of CMTs in cambium stem cells after ablation (Figure 5.3C) due to the, at least partial, removal of tissue-derived stresses, leaving mostly shape-derived mechanical stress (Figure 5.1B) which is in transverse direction. Due to this apparent competition, we assume that CMTs at the developmental stage considered here, are in a reorientation process from the rapid elongation towards radial growth of the hypocotyl. This transition is reflected

by a change from transverse to a longitudinal/oblique CMT orientation as observed in our control samples of the *in planta* cell ablations (Figure 5.5.1C, left; Video S1). Therefore, CMT orientation in non-perturbed samples cannot serve as a direct stress readout. For this, changes upon mechano-perturbation such as cell ablations need to be considered (Figure 5.5.1C, right). As a more definitive tissue readout, we considered changes of the mechanical environment described in the section after next.

Division rules are a key factor in tissue organization and division plane orientation

Having found support for the conclusion that our simulations faithfully captured tissue-wide stresses in the hypocotyl, we next employed our pre-simulated tissue mesh ($t = 5.0$, Figure 5.3B) to investigate the impact of cell divisions on tissue morphogenesis. To this end, we compared three different rules for cell division orientation: **i)** cells divide along the direction of maximum stress, **ii)** cells divide with random orientation, and **iii)** cells divide along their shortest axis (Cell division). For all cases, we imposed the following constraints: Only cambium cells were allowed to divide, but among those, cells were picked randomly for initiating cell division. As cambium cells, we considered cells in a radial range of $r_{min}/R \pm 0.075r/R$, where r_{min} was the minimum of the area distribution, corresponding to the center of the cambium domain. Due to the faster phloem growth as compared to the cambium, the local minimum of the area distribution was shifted towards the organ centre (Addendum Figures 5.6F, 5.8B,D,F). To ensure that only cambium cells divided during tissue growth, we tracked the minimum of the area distribution to dynamically keep the same reference for cell divisions throughout the simulation. In addition, cells only divided if they exceeded a certain size threshold, which was half of the average cambium cell size at the beginning of the simulation ($t = 0$). This rule was based on experimental data (Figure 5.1C) showing that cambium cell size remains on average largely constant, suggesting an upper size limit. Also, there was always only one division event per step and divisions were carried out only every 20 integration steps ($\Delta t = 0.2$). These rules ensured tissue growth in addition to the divisions and allowed the tissue to integrate effects of one division before possibly undergoing another one in their nearest neighbors. Simulations following the three different rules generated different cellular patterns. When cells divided in parallel to the maximum stress, division planes oriented largely periclinally at the beginning of each simulated division following the previously observed stress pattern (Figure 5.3B). When dividing randomly, resulting division planes generated angles with a uniform distribution. For dividing along the shortest cell axis, division planes were determined by taking the eigenvector of the smallest eigenvalue of the shape matrix M of the mother cell (cf. Section Cell division).

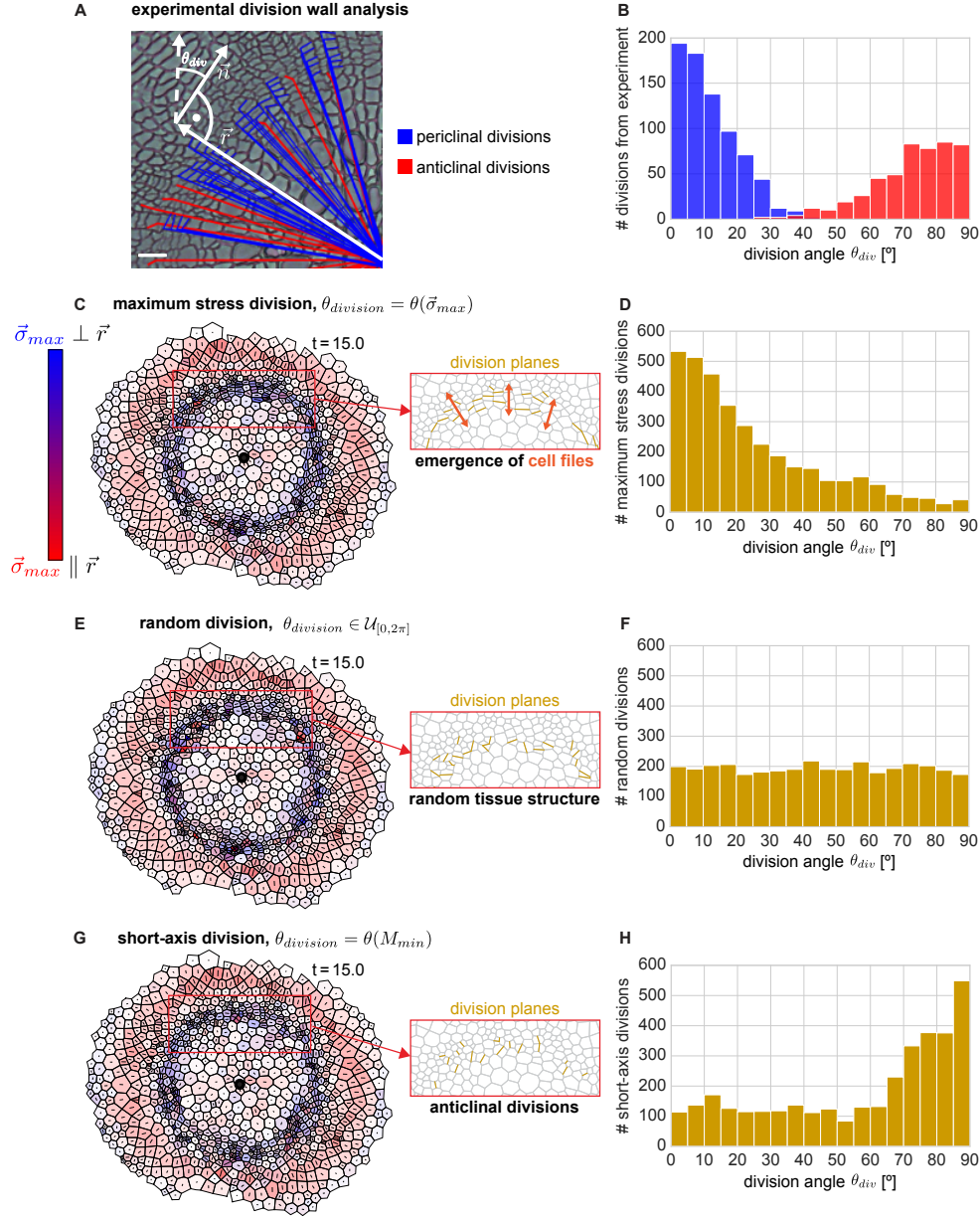


Figure 5.4: Experimentally measured peri- and anticlinal divisions correlate to maximum stress and short-axis divisions (A) Depiction of experimental acquisition of division plane angles w.r.t. the normal of the radius vector $\vec{r}$. Scalebar 10 μm (B) Histogram of the division plane angles θ_{div} as measured in (A) with periclinal (blue) and anticlinal (red) divisions. 15 independent hypocotyls cross-sections were analysed and a final database of 751 and 498 division angles was produced for periclinal or anticlinal cell divisions, respectively. 28-day-old seedlings were used to do the histological analysis. (C,E,G) Representative examples of tissues after 50 cell divisions with the maximum stress, random, and short-axis division rule, respectively. The insets show a magnification of parts of the cambium with division planes indicated in yellow. Cell files only emerge in the case of maximum stress division (C,D). (D,F,H) Histograms of division angles θ_{div} in direction of maximum stress, random direction and short axis direction respectively, with the angle being w.r.t. the periclinal direction, i.e. normal to the radius vector. Displayed is an average over 70 independent simulation runs. Details about the division angle quantification in (A,B) in the STAR*Methods. Details about the quantitative stresses, area distributions and stress variability in Addendum Figure 5.8.

In both our Voronoi meshes and experimental data, application of this rule resulted largely in anticlinal divisions (Addendum Figure 5.6B-D). As we were especially interested in tissue topology and cell division orientation, we compared these features generated under the different division rule regimes (Figure 5.4) with experimental data (Figure 5.4A,B). Interestingly, stress patterns in our simulations remained largely intact independently from the chosen division rule (Figure 5.4C,E,G and Addendum Figure 5.8A,C,E) indicating that not cambium stem cell behaviour but the faster growth of xylem and phloem tissue compared to the cambium (Figure 5.1C) was the driving factor for the emergence of these patterns. Yet, comparing stress orientation of daughter cells (Addendum Figure 5.8G-I), fluctuations were higher for random cell causing more local perturbation of the stress field. For our comparisons, division angles were defined as angles between the division planes and the direction $\vec{n}$ normal to the radius vector. Those planes were measured from one edge of an individual cambium cell to the centroid of whole cross-sections (Figure 5.4A). Quantitative analysis of division angles in plants (Figure 5.4B) showed that cambium stem cell divisions typically occurred either with a periclinal (0° w.r.t. radius vector normal $\vec{n}$) or anticlinal orientation (90° w.r.t the radius vector normal $\vec{n}$). Division plane orientations in the simulations were analyzed in the same way (Figure 5.3A). When doing so, we found striking differences between the different division rule regimes with regard to tissue topologies and distributions of division angles (Figure 5.4D,F,H). For divisions along the maximum stress, we mostly obtained periclinal (Figure 5.4D) divisions and it was only for this division rule that we observed the highly regular cell arrangement with emerging cell files as observed in plants. The large variation in the angles, reflected by the broadness of the histogram distribution, arose from local perturbations in the stress pattern and shape anisotropies. These may have led to high strains and stresses in individual cells, imposed by or imposing onto neighboring cells, which did not necessarily align with the overall stress pattern of the tissue. When letting cells divide randomly, we observed a uniform distribution of division angles and a rather irregular arrangement of cells (Figure 5.4F). For short-axis divisions, anticlinal divisions were clearly the most prominent category (Figure 5.4H) and there was no clear topological pattern emerging. While random divisions showed, as expected, a uniform distribution, short-axis divisions lead to an angular distribution that was complementary to the result of maximum stress divisions. This was a result of the short axis of cells pointing almost exclusively in radial direction which was largely perpendicular to the direction of maximum stress (Addendum Figure 5.6C,D).

In conclusion, while all division rules left the stress pattern intact, only divisions along the direction of maximum stress led to the emergence of radial cell files in cambium-derived tissues as observed in plants. Therefore, our results demonstrated that divisions along the

direction of maximum stress are strong candidates for directing cell divisions in cambium stem cells and highlight the significance of mechanical forces towards tissue morphogenesis. As an interesting consideration, a combination of the maximum stress and short-axis division rules may serve as an explanation for the distributions of division plane orientations that were observed in plants. In that sense, mechanical stress may be the factor guiding cell file emergence, while the geometric short-axis rule is a candidate for guiding anticlinal divisions increasing the cambium stem cell pool in circumferential orientation.

Mechano-perturbations lead to similar tissue organization changes *in silico* and *in planta*

To probe the significance of the alignment of cambium stem cell divisions along the direction of maximum stress, we investigated divisions and vascular tissue organization in hypocotyls under exogenous mechanical stress. Therefore, we constrained hypocotyl expansion by positioning a clip applying pressure from two opposite sides of the hypocotyl after four weeks of non-perturbed growth (Figure 5.5A and Addendum Figure 5.9A). As the organ continued to expand, growth was thus constrained in two directions, resulting in regions with apparent anatomical differences after three days of treatment (Figure 5.5A, right, white dashed lines). The regions of sections which were positioned adjacent to the clip showed a very regular tissue structure, similar to the structure observed under regular growth conditions (Figure 5.1A) and harbored typical radial cell files (Figure 5.5C,a). In comparison, in the regions which were positioned perpendicular to the clip (Figure 5.5C,b) tissue anatomy was very chaotic and no cell files were present. The more regular tissue anatomy next to the clip was not due to a general suppression of cambium stem cell divisions under the physical confinement, though, because the activity of *pHTR13::pHTR13-mCherry* reporter indicating S-phase and early G1 phase during cell divisions and part of the PlaCCI (plant cell cycle indicator) reporter²⁴³ was comparably active under perturbed and non-perturbed conditions (Addendum Figure 5.9B-D). Hence, the high regularity of division plane orientation in cambium stem cells adjacent to the clip did not simply reflect the situation before the start of the treatment. We further checked cambium organisation under clip treatment using combined *pPXY* and *pSMXL5* reporters visualizing cambium organization⁴² and found that the typical arrangement of cambium domains was highly disturbed in regions positioned perpendicular to the clip (Addendum Figure 5.9E,F). Since CMT orientation is parallel to the maximum stress direction and directly influences cell division orientation²⁴⁴, we next checked CMT organization in cambium stem cells in the different regions of perturbed hypocotyls. These analyses showed that CMTs were more isotropic in cambium stem cells at positions perpendicular to the clip possibly reflecting

an altered physical environment in the regions (Addendum Figure 5.10A,B). In simulations, we used the same initial mesh as for the previously studied cell divisions (Figure 5.3B) and applied a confining repulsive, external potential $U(r/R)$ in the shape of a logistic function (Eqn. 5.11). This confined growth in x-direction analogously to the *in planta* experiments, allowing growth in y-direction only (Figure 5.5B). When applying again the maximum stress cell division rule

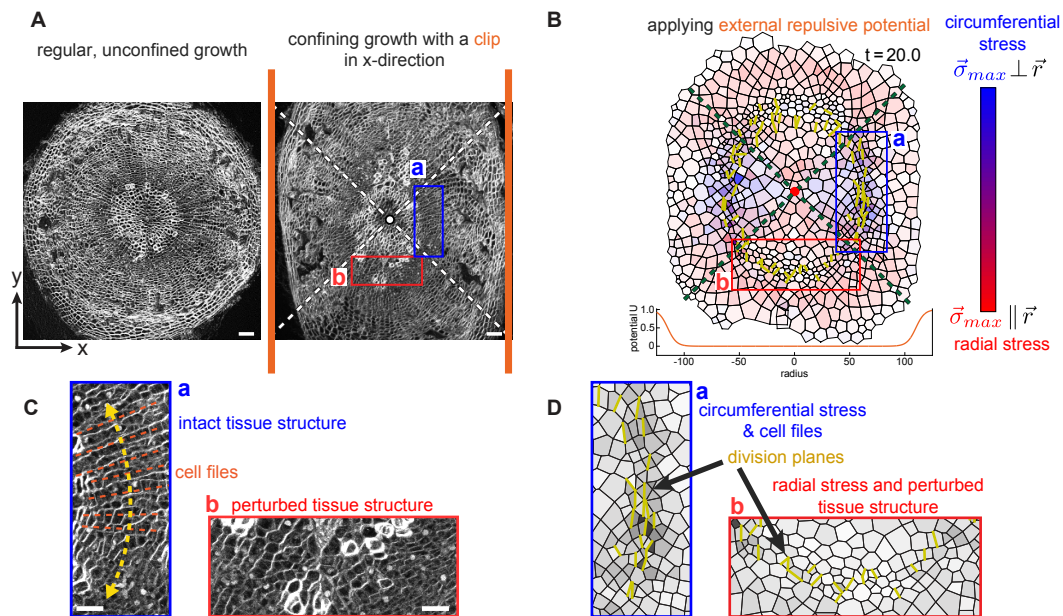


Figure 5.5: External mechanical stress instructs cambium organization by perturbation of division wall orientation (A) cross-sections of hypocotyls under regular, unconfined growth condition (left) and under exogenous mechanical stress through confinement by clip treatment in x-direction (right). White lines correspond to quarters of tissue where the tissue structure remains well-organized (a, and directly opposite tissue quarter) or where the tissue structure is perturbed, respectively (b, and directly opposite tissue quarter). 10 biological replicates were carried out. 31-day-old seedlings were used to do the histological analysis. Scalebar: 50 μm (B) Implementation of the clip treatment in simulations. Growth is confined in x-direction by adding an external potential of logistic shape, acting as a soft wall on both sides of the simulated tissue. Shown is the tissue from Figure 5.3B after growth within the external potential ($t = 20$) and 75 cell divisions following the maximum stress rule. (C) Magnifications of cross-section (A, right) with intact tissue structure including cell files (a) and perturbed tissue structure (b). Scalebars: 25 μm (D) Magnifications of two sections of the simulated tissue. Yellow lines represent the division planes in the cambium. Division planes are oriented almost exclusively in y-direction as a result of the applied external potential, overriding the original stress pattern of the tissue in b and the quarter directly opposite of b (Figure 5.3B). This is accompanied by an intact tissue structure and the occurrence of cell files in D,a and a perturbed structure in D,b. Details on the experimental result regarding the impact of the clip treatment on cell division rates and cambium organization in Addendum Figure 5.9. Details on the CMT orientation in compressed versus non-compressed regions and growth anisotropy in simulations under confinement in Addendum Figure 5.10. Details on the external potential in the STAR*Methods.

for 75 divisions under these conditions, we observed largely disrupted stress patterns in the regions positioned perpendicular to the confinement. After few steps of simulation, stresses started to realign towards the y-direction, disrupting the concentric pattern of radial stress.

Monitoring the role of individual cell growth anisotropy (Addendum Figure 5.10C-H) we observed a slight redirection from the radial to the y-direction, emerging first in cells being directly subjected to the repulsive, external potential and over time propagating through the tissue and thus step-by-step deforming the tissue as a whole. As in the *in planta* experiments, we divided the tissue in four quarters (Figure 5.5B, green dashed lines). Cambium stem cells within the two quarters being directly subjected to the external constraint effectively remained under circumferential stress (Figure 5.5D,a) while cambium stem cells located in the two quarters perpendicular to the exogenous stress were under radial stress (Figure 5.5D,b). For the two quarters maintaining circumferential stress, the simulation revealed almost unchanged stress patterns compared to our previous simulations manifested by the emergence of radial cell files due to periclinal cell divisions. On the contrary, for the two quarters in which cambium stem cells experienced radial stress (Figure 5.5D,b) anticlinal cell divisions emerged preventing cell file formation very much in accordance with the *in planta* results. Therefore, we concluded that our simulations faithfully reported stress patterns in radially growing hypocotyls and that there is a functional connection between tissue organization and organ-wide mechanical stress patterns which emerge through a combination of locally defined cell production and expansion rates.

5.4 Discussion

Radial growth makes a vital contribution to the production of biomass on earth, however an in-depth understanding of the regulation of cell divisions and the influence of different mechanical environments is yet unknown. In this study, we combined *in planta* experiments and *in silico* simulations to understand how mechanical stress regulates cell division plane orientation in cambium stem cells and general tissue organization in radially growing plant organs. We developed a cell-based vertex model (Box 1) based on experimental recordings of hypocotyl growth (Figure 5.1C) in order to study mechanical stress patterns and their morphological effects on the emerging tissue structure. This endeavor was achieved by taking into account experimental evidence to derive rules for cell growth rates and estimate initial conditions, including realistic meshes from segmentations. When applying these rules in simulations, we observed a rapidly emerging characteristic stress pattern (Figure 5.3B) with high circumferential stress in cambium stem cells as a result of fast growing cells surrounding the stem cell tissue, possibly straining and thereby stressing cambium stem cells accordingly. While in earlier work on stems, an impact of the epidermis on the mechanical properties of the inner tissues has been shown^{48,245}, we found the cambial stress pattern

in our simulations of radial hypocotyl growth to be very robust and largely independent of mechanical changes (Addendum Figure 5.7A-D). Regarding cell geometry, whereas in plants and in simulations the short-axis orientation of cambium cells was largely in anticlinal direction, the experimentally obtained division planes were mostly in periclinal direction. This discrepancy already hinted at a possible correlation between mechanical stress and cell division plane orientation, rather than divisions being guided by cell geometry alone. In simulations, we compared three different division orientation rules: maximum stress, random and short-axis. We observed that in all cases the stress pattern emerging during growth (Figure 5.3B) was the same and, thus, independent of the applied rules (Figure 5.4C-H). In contrast, only when applying the maximum stress rule we found tissue morphologies in agreement with *in planta* morphologies characterized by cell files emerging from mainly periclinal cell divisions. Periclinal divisions were also present in externally compressed hypocotyls. Specifically, compressed regions exhibited periclinal divisions in simulations and *in planta* experiments producing well-structured cambium-derived tissues with emerging cell files. In contrast, organ regions experiencing tensile stress exhibited anticlinal divisions and a comparatively distorted tissue morphology again in simulations and in plants. These findings strongly argue for a connection between tissue-wide mechanical stress and the orientations of cell divisions and a resulting instructive role of mechanical stress in tissue morphogenesis during radial growth.

Yet, it appears that geometry still affects division plane orientation as there was a high amount of anticlinal divisions observed in plants (Figure 5.4B) resembling short-axis-driven divisions observed in simulations (Figure 5.4H). This suggests that on the one hand, mechanical stress largely guides cell divisions and leads to highly ordered tissue structures through the emergence of radial cell files. On the other hand, short-axis-driven divisions may be part of the collection of divisions found in cambium stem cells. Finding the lowest stress variability in daughter cells after division in our simulations for short-axis divisions (Addendum Figure 5.8G-I) could be an additional clue towards a synergy here. It bears mentioning that the term 'short-axis' applies only when looking at the cambium in two-dimensional cross-sections. Because cambium stem cells are very much elongated along the longitudinal organ axis, the shortest cell axis in fact lies perpendicular to this axis. Still, taking into account the aforementioned considerations, we suggest a combination of stress-based and geometry-based cell divisions in the cambium. As our considerations imply that at first order, longitudinal stress overall dominates, leading to the long-axis divisions experimentally observed in cambium stem cells, we propose mechanical stress to likely be the main instructor

for division plane placement in the longitudinal direction. Then and at second order, in transverse plane, we propose the orientation of the division plane to be guided either as well by stress anisotropies, i.e. in periclinal direction or by geometry, i.e. in anticlinal direction. To further test our conclusion for instance further perturbations of the tissue mechanics for example via hormones could be instructive as well as full three dimensional modeling of the entire organ.

As cambium stem cells are embedded deeply in growing organs, it is so far almost impossible to directly visualize them alive in their natural environment. Advanced experimental techniques like combined Raman and Brillouin microscopy⁶⁵ allow for the non-invasive acquisition of quantitative data about physical properties of tissues. Yet, due to limitations in light penetration it is also not possible for these techniques to collect physical data on cambium stem cells in a natural non-perturbed context. Consequently, we believe that the simulation platform generated during this study will serve as a highly useful tool for investigating mechanical stimuli in the context of radial plant growth. Getting a better understanding of tissue growth and division in the hypocotyl and other tissue types, especially the impact of mechanical stress towards these, will provide more insight into plant growth organization under specific environmental conditions. Based on our results, we assume that centripetal tissue production by peripheral stem cell systems, like in the root or in primary vascular bundles of the inflorescence stem, may generate similar organ-wide forces and explain in general the dominance of periclinal cell divisions found in cambium stem cells in these contexts^{246,247}. This could be a valuable insight for agriculture and forestry, helping to increase fitness and resistance of growth in diverse ecological settings for example where mechanical stress might be a limiting factor, such as windy or rainy environments or the influence of gravity²⁴⁸. With this work it furthermore becomes apparent that not only in the shoot apical meristem but also in other plant tissue, mechanical stresses play a major role in tissue organization and the production of biomass via influencing cell divisions.

Resource availability

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact Karen Alim (k.alim@tum.de)

Materials availability

This study did not generate new unique reagents.

Data and code availability

The data collected and simulation code used for this study is publicly available online at: <https://doi.org/10.14459/2024mp1741765> as of the date of publication. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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Author contributions

Conceptualization, all authors but especially K.A. and T.G.; Methodology, all authors but especially K.A. and T.G.; Software, M.H.; Validation, M.H. and X.L.; Formal Analysis, M.H. and X.L.; Investigation, M.H., X.L., T.G. and K.A.; Resources, T.G. and K.A.; Data Curation, M.H. and X.L.; Writing - Original Draft, M.H., X.L., T.G. and K.A.; Writing - Review & Editing, M.H., T.G. and K.A.; Visualization, M.H.; Supervision, T.G. and K.A.; Project Administration, T.G. and K.A.; Funding Acquisition, T.G. and K.A.

Declaration of interests

The authors declare no competing interests.

5.5 Addendum: Supplemental information

5.5.1 Methods

STAR★METHODS

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 - External potential
- QUANTIFICATION AND STATISTICAL ANALYSIS
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 - Cell division angles quantification

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Plant material and culture conditions

Arabidopsis thaliana of Col-0 ecotype was used throughout this study. Arabidopsis seeds were surface sterilized with 70 % EtOH and 0.05 % Triton X-100 (Sigma, T8787) and shaken for 1 or 2 minutes on a rotating wheel, the liquid was removed by a Pasteur pipet and 200 to 300 µl 100 % EtOH was added. Next, seeds were dried for one minute on a filter paper under sterile conditions. Sterilized seeds were sown onto sterile agar plates containing 1/2 Murashige and Skoog (MS) solid medium supplemented with 1 % sucrose, and stratified at 4°C for 2 days in the dark. Seedlings were transferred from plates

to soil after 1 week in growth chambers under short day (SD) conditions (10 h light, 4 h dark, 22°C, 65 % humidity). 3-week-old seedlings were moved from SD to long day (LD) conditions (16 h light, 8 h darkness, 22°C, 50 % humidity).

Vector construction

For *pWOX4::mVenus-MBD* (*pXL12*), the following modules were combined in pGGZ001: WOX4pro(A), B-dummy(B), mVenus(C), MBD(D), WOX4 terminator(E) and hygromycinR(F)^{42,249–251}.

Histology

4-week-old hypocotyls embedded in JB4 resin (Polysciences, 00226-1) were used for the histological analysis (Figure 5.1A) as previously described²¹⁷. 5 µm thick sections were made and visualized using bright field light. Images were captured by a Leica DM IRB microscope. The images shown in Figure 5.2A and Figure 5.5A; 5.5C are free-hand hypocotyl cross-sections. For this, hypocotyls were embedded in 8 % Low Melting agarose (Sigma-Aldrich, A9414) in a small plastic weighing cup and cut with a razor blade (Wilkinson Sword). Sections were then put in a 2-well glass-bottom dish (ibidi, 80287) and stained with 0.1 % Direct Red 23 (Sigma-Aldrich, 212490) in Ca²⁺ Mg²⁺-free PBS for 5 min. After washing with water once, images were captured.

METHOD DETAILS

Confocal microscopy

A Nikon A1 confocal microscope with a 25× water immersion objective lens (Nikon, Apo 25xW MP, 77220) was used to capture Figure 5.2A. The 561 nm laser was used to excite Direct Red 23. Hypocotyls of 2-week-old WT seedlings on plates under SD conditions were used. Images were collected at 2048×2048 pixel resolution and 4× line average. A Leica Stellaris 8 confocal microscope with 20× air objective (HC PL APO CS2, 20X/0.75 DRY) or 63× water objective (HC PL APO CS2, 63×/1.2 WATER) and were used in all other images in this study. The Diode 405 nm and WLL lasers were used for excitation of fluorescent proteins or dyes. GFP was excited at 473 nm and detected at 480 nm to 529 nm. Citrine and mVenus were excited at 514 nm and detected at 558 nm to 615 nm. Autofluorescence of lignin in xylem vessels was visualized using a 405 nm laser and the emission was collected at 425 nm to 474 nm. Direct Red 23 was excited at 495 nm and detected at 558 nm to 615 nm. Image acquisition was performed at 1024×1024 pixel scanning field and 4× line accumulation.

Image and data analysis

The image in Figure 5.2A was processed as previously described to get the cell mesh⁴⁴. The PlantSeg parameters were set as follows: prediction patch size-1×128×128, stride-Accurate, segmentation algorithm-

MutexWS, under-/over-segmentation factors-0.45, CNN, prediction threshold-0.06, Watershed seeds sigma-4.2, Watershed boundary sigma-4.2.

Cell ablation

The hypocotyl surface was exposed by cutting plants at the hypocotyl-root border. Next, the xylem tissue was removed using a needle. In controls, the hypocotyl surface was exposed but that xylem was left intact. Plants were then left recovering for one day in 1/2 MS liquid medium in growth chambers under LD conditions and free-hand sections using a razor blade (Wilkinson Sword) were produced . Those were immersed in water in a 2-well glass-bottom dish (ibidi, 80287). Related to Figure 5.3C.

Clip treatment

Home-made clips were applied to the upper parts of hypocotyls of 4-week-old seedlings grown in soil. After three days, clips were carefully removed and hypocotyls collected for histological analysis. Related to Figure 5.5, Addendum Figures 5.9 and 5.10.

Simulation framework and parameters

We use a C++ code, based on the quad-edge²⁰⁰ data structure and its implementation as a C library²⁰¹. The energy function (Eq.5.5) is minimized by using the C library NLOPT²¹² with the NLOPT_LD_VAR1 algorithm, which is based on²¹³. ODEs in our model are solved with the GNU Scientific Library (GSL)²¹⁴, using the integrated Runge-Kutta-Fehlberg method²⁵² (RKF45). Simulation parameters are determined as far as possible from the literature. Consulting^{46,66,85}, we apply a reference stiffness of $E_0 = 300$ MPa and a turgor pressure of $P = 0.5$ MPa. The Poisson ratio we set to $\nu = 0.3$.

These parameters are connected to the Lamé constants in the model description of the previous section via the following conversions, based on classical continuum mechanics^{160,202}:

$$\mu = \frac{E_0}{2(1 + \nu)}$$

$$\lambda = \frac{E_0 \nu}{(1 + \nu)(1 - 2\nu)}.$$

Similarly to²³, for cell ablation we set the stiffness of ablated cells to $E_y = 0$ and set a residual turgor pressure to ten percent of the original value, i.e. $P = 0.05$ MPa.

The simulation time we estimated from the tissue radius over time (Addendum Figure 5.6K), comparing with experimental data, resulting in division simulations to correspond to two days of real-time and clip treatments corresponding to three days in real-time.

Vertex model

Simulations employ a cell-based vertex model as introduced in⁶⁷ and furthermore applied in^{68,88,89} that allows individual access to the mechanical properties of each cell within the tissue. A cell is represented as a pressurized container following the dynamics of a linearly elastic isotropic solid. Tissues used for the simulations are derived from experimental data, segmented with the software PlantSeg/MorphographX^{91,211}. The segmentation is used to obtain cell centers which themselves determine the Voronoi tessellations that serves as initial condition for simulations. Abstracting the recorded tissue by a Voronoi tessellation maintains the experimentally recorded cell area distribution (Addendum Figure 5.6A), yet balances the accuracy of tissue representation and numerical robustness as using the segmentation would require taking into account an amount of order 10^5 cell walls, exceeding our computational resources.

Stress and strain from shape matrix description

Individual cells are described by shape matrices M and M_0 (Figure 5.2C). M_0 hereby represents the shape before deformation, i.e. the shape that minimizes the tissue energy $\mathcal{H}$ (Eq. 5.5) and M represents the shape after deformation defined by the second moment of area which in index notation is^{67,88}:

$$M_{ij} = \iint_V (x_i - X_i)(x_j - X_j) dV, \quad (5.1)$$

where x_i are the vertex coordinates of the cell and X_i the coordinates of the center of the cell. The formalism of employing the current shape M and the rest shape M_0 allows to derive individual cell strain²⁰²

$$\begin{aligned} \varepsilon = & \frac{M - M_0}{\text{Tr}(M_0)} - \frac{1}{2} \frac{M_0}{\text{Tr}(M_0)} \frac{\text{Tr}(M_0) \text{Tr}(M - M_0) - M_0 : (M - M_0)}{\det(M_0)} \dots \\ & - \frac{1}{2} I \left(\frac{\text{Tr}(M - M_0)}{\text{Tr}(M_0)} \right) + \frac{3}{8} I \left(\frac{\text{Tr}(M_0) \text{Tr}(M - M_0) - M_0 : (M - M_0)}{\det(M_0)} \right). \end{aligned} \quad (5.2)$$

Strain determines stress from the constitutive relation

$$\sigma = 2\mu\varepsilon + \lambda I \text{Tr}(\varepsilon), \quad (5.3)$$

with Lamé parameters μ and λ . Cells grow exponentially by plastic deformation^{67,155,253}, driven from cell shape mismatch before and after deformation, M_0 and M , respectively. Similarly to⁶⁷, M_0 evolves as:

$$\frac{1}{\text{Tr}(M_0)} \frac{dM_0}{dt} = \kappa(r/R)\varepsilon - \eta D \quad (5.4)$$

where the first term $\kappa(r/R)\epsilon$ represents strain-based growth with a cell-type specific growth factor here determined by a cell's relative radius r/R . The second term models mechanical feedback mediated by CMT alignment with feedback constant $\eta = 0.01$ and the deviatoric stress tensor D which enhances growth in directions perpendicular to the maximum stress and reduces growth in the directions parallel to it as determined from the eigendecomposition of the stress tensor σ .

The total energy is described by^{67,194,202} (see Box 1):

$$\mathcal{H} = \frac{1}{2} \sum_{a=1}^n A_{0,a} h (2\mu \text{Tr}(\epsilon^2) + \lambda [\text{Tr}(\epsilon)]^2) - \sum_{a=1}^n P A_{0,a} h, \quad (5.5)$$

where the first sum is the elastic energy of the cells in the tissue and the second sum is the turgor pressure term, where A_0 is the cell area in the undeformed state and h the cell height and equals one here since our model is strictly two-dimensional.

Cell division

Cell division always occurs through the centroid of the dividing cell. For random division, cells divide along a random direction chosen from a uniform distribution (Figure 5.2E). For maximum stress divisions, we obtain the division plane orientation from the largest eigenvalue of the eigenvectors of the stress tensor σ . For short-axis division, we compute the eigenvector corresponding to the smallest eigenvalue of the cell's observed shape matrix M . Upon cell division, we keep the strain of the mother cell conserved by daughter cells inheriting their M_0 from the dividing mother cell. Since strain and stress directly depend on the current shape M and rest shape M_0 , we derive the new daughter cells' rest shapes after division by transformation. As strains are very small, we transform between the current, deformed matrix M and the undistorted rest shape M_0 with a single transformation Λ , employing the definition of infinitesimal strain from the displacement gradient. That is²⁰²

$$M_0 = \Lambda^{-1} M (\Lambda^{-1})^T \det(\Lambda^{-1}), \quad \text{with} \quad (5.6)$$

$$\Lambda = \mathbf{1} + \epsilon, \quad (5.7)$$

where Λ^{-1} is determined from inversion of Λ by

$$\Lambda^{-1} = \frac{1}{\det(\Lambda)} \begin{pmatrix} \Lambda_{yy} & -\Lambda_{xy} \\ -\Lambda_{xy} & \Lambda_{xx} \end{pmatrix}. \quad (5.8)$$

It then holds for all, the dividing mother cell and the resulting daughter cells, that

$$M_0^M = \Lambda^{-1} M^M (\Lambda^{-1})^T \det(\Lambda^{-1}), \quad (5.9)$$

$$M_0^{D_i} = \Lambda^{-1} M^{D_i} (\Lambda^{-1})^T \det(\Lambda^{-1}), \quad (5.10)$$

with the same matrix Λ^{-1} . This linear transform directly conserves the total strain in the system since it is just transferred to both daughter cells with the same transformation, thus also conserves total energy.

External potential

For the application of an external potential (Figure 5.5B), we add a term U to the energy function (Eq. 5.5). For this we choose a logistic curve as it's continuously differentiable and allows for a big slope to properly confine simulated tissue. This is defined as:

$$U_i = \frac{a}{(1 + \exp((x_i - c_1)b))} + \frac{a}{1 + \exp(-(x_i - c_2)b)}. \quad (5.11)$$

where U_i is the contribution to the energy by a vertex at position x_i .

QUANTIFICATION AND STATISTICAL ANALYSIS

The precise statistics for each figure can be found in the respective figure's caption.

CMT quantification in cambium stem cells

Longitudinal sections of *pWOX4::mVenus-MBD* hypocotyls were used in this experiment. Pixel size in z dimension was set to 1 μm . CMTs organization of individual cambium cells was detected by choosing different stacks of images and making max projections in FIJI²⁵⁴. Microtubules orientation and anisotropy of the selected cambium stem cells were taken from the 'FibrilTool' in FIJI as described before²⁵⁵. Related to Figure 5.3E and Addendum Figure 5.10B.

Cell division angles quantification

Images taken from JB4 resin sections were used to quantify cell division orientation in the cambium. FIJI was used to conduct images analysis by using the 'angle tools'. For periclinal cell divisions, angles from the centroid of cross-sections to the tangent of the cell file (no less than 3 cells) direction were measured. Angles of anticlinal cell division were indicated by the angle between the centroid of cross-sections and the common cell wall of two cells which break up cell files. 15 independent hypocotyls cross-sections were analysed and a final database of 751 or 498 division angles was produced as periclinal or anticlinal cell divisions, respectively. Related to Figure 5.4A,B.

5.5.2 Supplemental figures

Video S1: Max projection shows helical orientation of CMTs. Related to Figure 5.3C.

3D movie showing max projections of cortical microtubules in cambium stem cells of the control (Figure 5.3C, left), using 38-day-old hypocotyl longitudinal sections. Step size=0.356 μm . Available here: <https://doi.org/10.14459/2024mp1741765>.

Supplemental figures:

Figure 5.6: Simulation of the tissue as voronoi mesh retains the quantitative features of the original tissue and applying the derived growth function (Figure 5.2D) leads to sharp change in stress orientation in cambium and desired growth evolution of the simulated tissue. Related to Figures 5.2 and 5.3. (A) Fit of the normalized individual cell areas A as a function of the normalized radius r/R for both the segmentation (yellow) and the derived Voronoi tessellation (grey) in comparison. (B) Angular orientation of short axis in simulation averaged and binned (bin size 0.033) as a function of the normalized radius r/R . (C) Voronoi mesh of simulation with short axes indicated (green lines). (D) Segmentation of 2 weeks old sample with indicated surface areas (colors) and short axes (white lines). (E) Evolution of averaged, binned (bin size 0.033) cell stresses. (F) Fit of individual cell areas as a function of the normalized radius r/R . For (B,E,F), the data was averaged over 100 independent runs. (G) Ashman's D^{239} for the experimental data in Figure 5.1C as a criterion that the average cell area distribution can be considered bi-modal. (H) Amplitudes of the Gaussian fits to the experimental data in Figure 5.1C with slopes $\alpha = 19.16$ and $\beta = 66.67$. (I) Means of the Gaussian fits to the same data with a slope of $\gamma = 0.0016$ and $\delta = 0.031$. (J) Gaussian fit (black) of the Voronoi mesh areas as in Addendum Figure 5.6A and the same fit function with adding the slopes from (A) and adjusting the variance to match the maxima and the local minimum of the Voronoi mesh areas. The difference of these curves results in the radial growth factor $\kappa(r/R)$, we apply to individual cell growth in our simulations. (K) From exponentially fitting the tissue radius, using the data from Figure 5.1C and Addendum Figure 5.6A, for division simulations (red star) shown in Figure 5.4, the simulated time of $t=15$ corresponds to real-time of two days and for the simulation of the clip treatment (green diamond), shown in Figure 5.5A the simulated time of $t=20$ corresponds to three days as in the experiment. (L) Simulation tissue mesh as Voronoi mesh with Manhattan metric, based on the segmentation of the tissue (Addendum Figure 5.6D). (M) Simplified version of the Voronoi mesh with Manhattan metric by removing cell “tails” and two-way junctions of cell walls. Cells maintain their rather rectangular shape, similar to the segmentation (Addendum Figure 5.6D). (N) Simplified Voronoi mesh with Manhattan metric under the same growth conditions as the tissue shown in Figure 5.3B. Despite some artificial cell shapes arising from the mesh simplification the observed stress pattern remains the same as with rather hexagonal shapes from Voronoi-meshing with Euclidean metric as used throughout the manuscript.

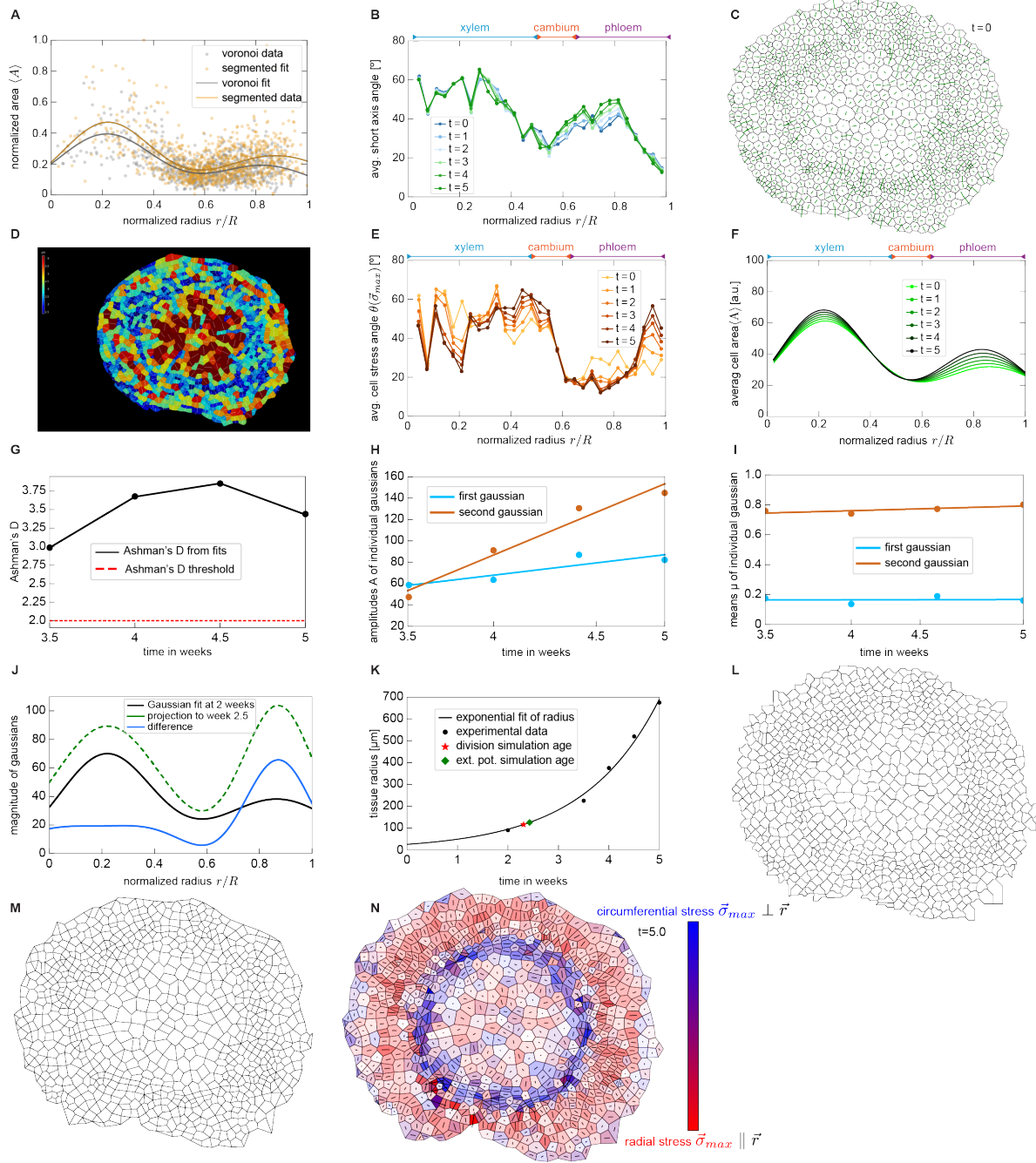


Figure 5.6

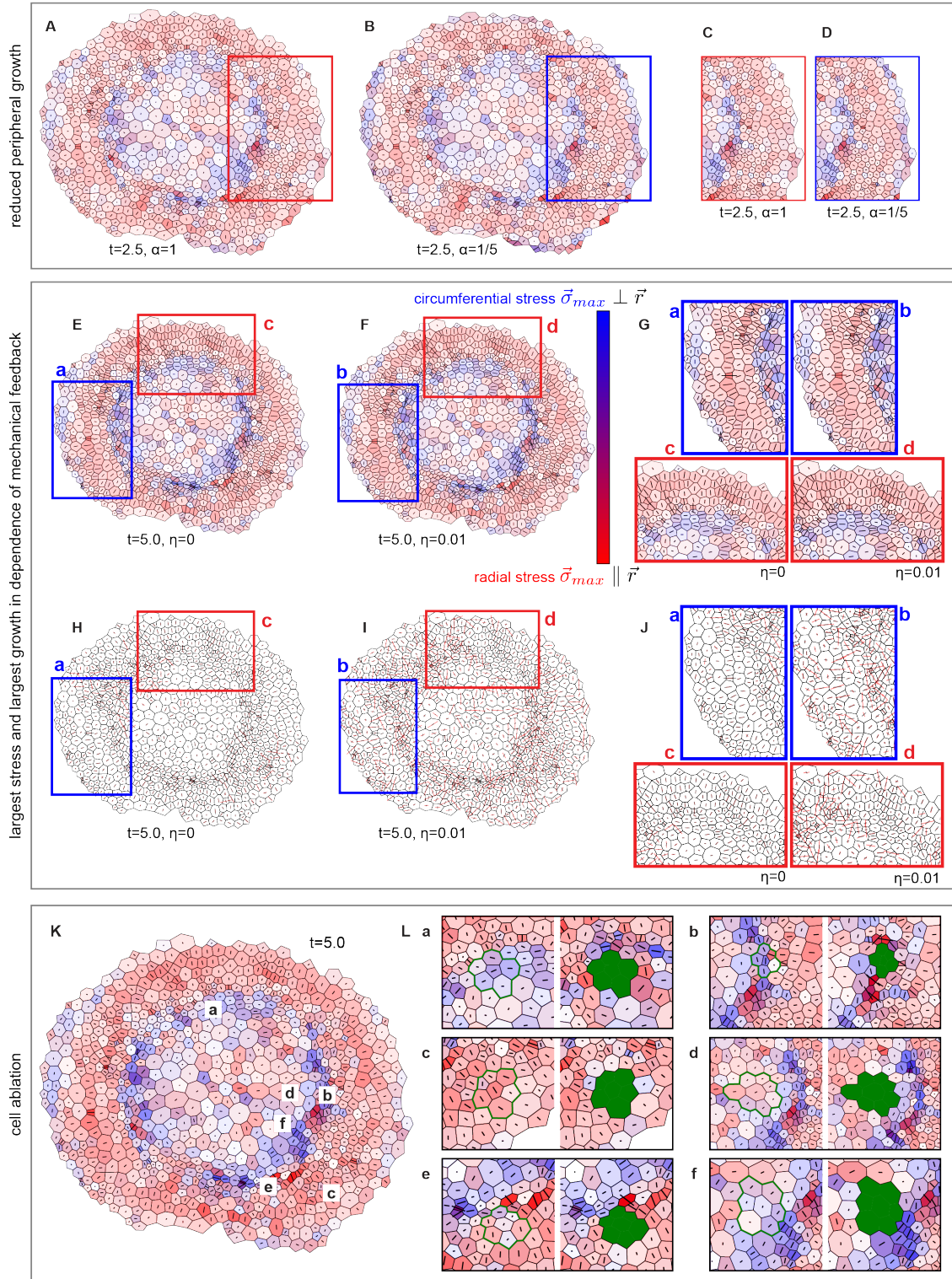


Figure 5.7

Figure 5.7: Reduced peripheral growth leads to circumferential stress, mechanical feedback controls the direction of growth and cell ablation leads to stress, circumferentially aligned around the wound. Related to Figure 5.3. (A) Tissue under standard growth conditions as in Figure 5.3B. (here at $t=2.5$). (B) Tissue under the same conditions as in (A) but with the peripheral cell layer having its growth reduced to 20% of the original value (additional growth modulation $\alpha = 1/5$ at the peripheral cell layer) in order to probe for a possible impact of changes from the epidermis towards the tissue stress. (C,D) Zoom-in of (A) and (B) showing the highlighted parts of the tissue, indicating slower growth at the peripheral layer to result in circumferential stress on the tissue boundary while leaving the stress pattern of the cambium virtually untouched. (E,F) Tissues under standard growth conditions as in Figure 5.3B. ($t=5.0$) but without mechanical feedback ($\eta = 0.0$) (E) and including mechanical feedback ($\eta = 0.01$) (F). (G) Zoom-in of (E) and (F) showing different parts of the tissue. (H,I) Tissues shown in (E,F) with indication of direction of largest growth (red lines) and maximum stress (black lines). (J) Zoom-in on (H,I), showing the largest growth to be oriented along maximum stress without feedback and orientations perpendicular to stress with the inclusion of mechanical feedback, leading to anisotropic cell growth. (Note that the scaling of the indicated stress anisotropy (black lines) is increased slightly here, compared to Figure 5.3B, to better visualize differences with and without feedback.) (K) Pre-grown tissue, identical to Figure 5.3B with ablation sites indicated by lower-case letters. (L) Ablation sites before and after ablation. The surrounding tissue response depends on the specific stresses of cells, however the ablation typically leads to overall increased stress, surrounding the wound. Especially if ablating the center of the cambium (a, b) a particularly strong response is found, indicating the compressive forces that the cambium experiences by xylem and phloem tissue.

Figure 5.8: Different division rules do not largely change the stress pattern or cell area distribution but do affect the probability density function of stress angles of daughter cells after division. Related to Figure 5.4. (A,C,E) Average stress angles w.r.t. radius vector $\vec{r}$ for random division, maximum stress division and short-axis division respectively at different time steps after the start of division ($t = 5$). (B,D,F) Fit of area distribution for all division rules at the same steps. (G,H,I) Probability density functions of stress angles of daughter cells after division, corresponding to the data shown in Figure 5.4, maximum stress division (G), for random division (H) and short-axis division (I). The least stress-angle confinement is observed for random cell divisions, indicating fluctuations of the stress pattern, while short-axis divisions conserve circumferential stress the best. For every plot, the data includes 70 independent runs with 50 cell divisions each.

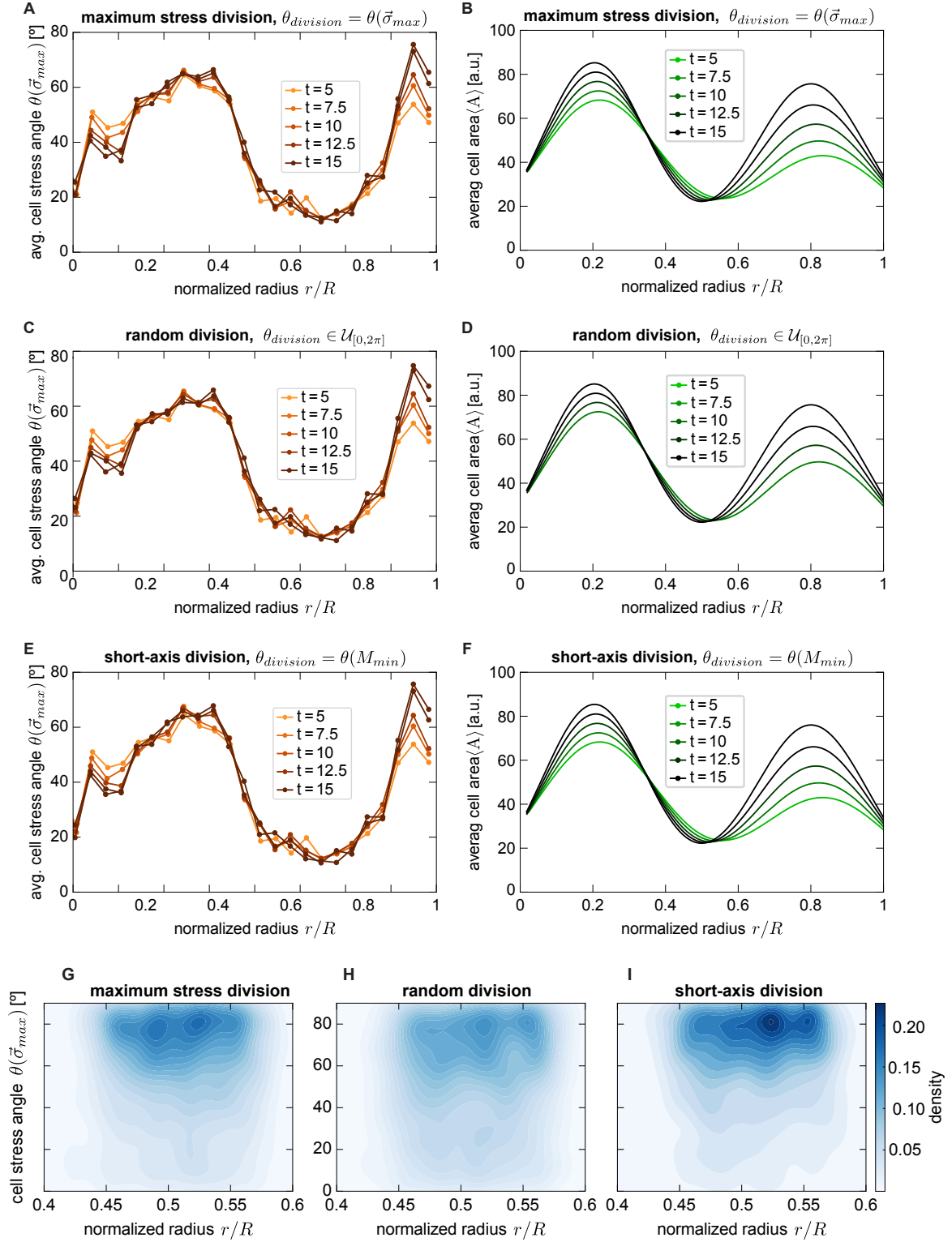


Figure 5.8

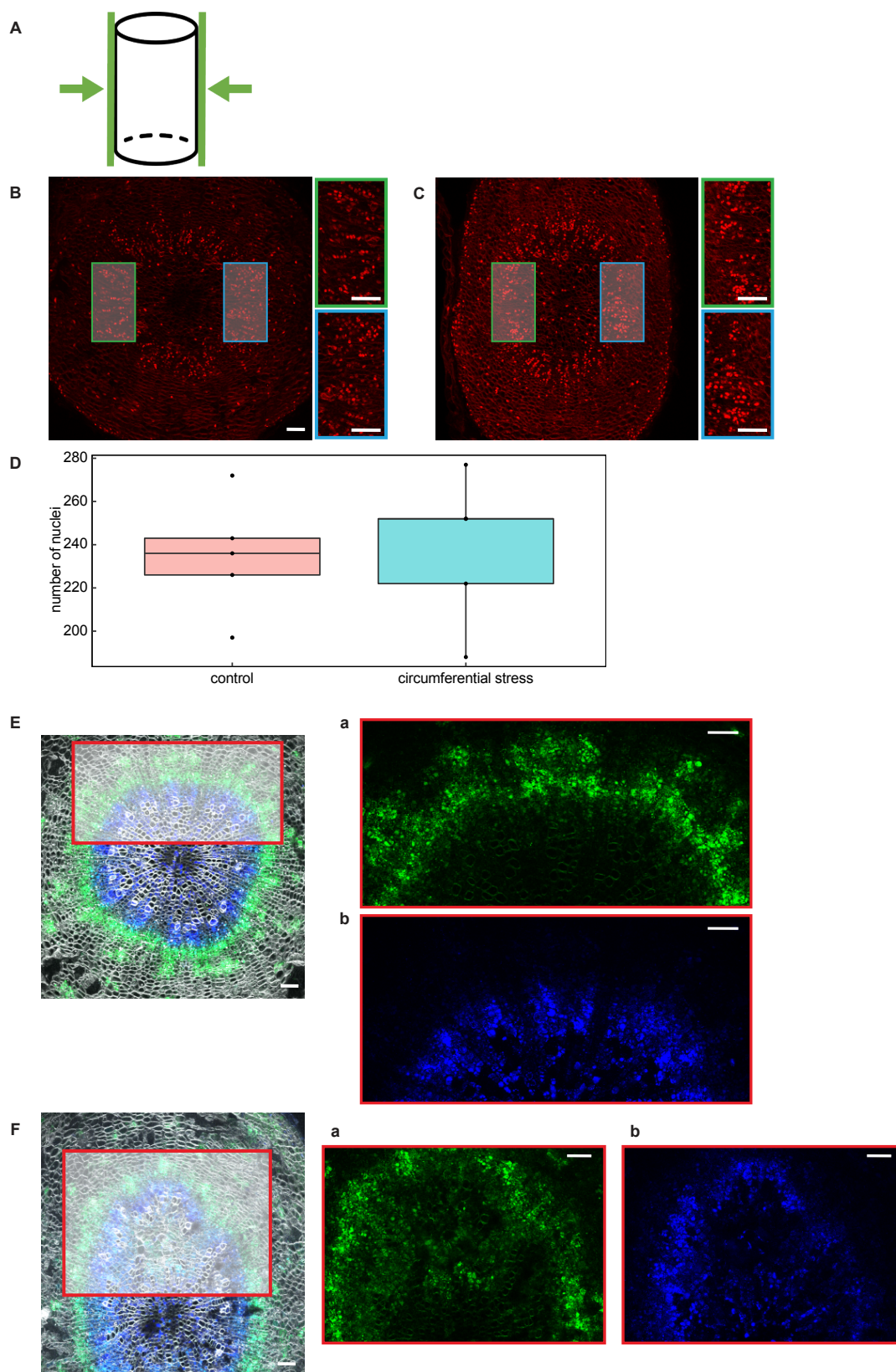


Figure 5.9

Figure 5.9: Effect of mechano-perturbation on cell division rates and cambium organization. Related to Figure 5.5. (A) Schematic figure of exogenous mechanical stress treatment of the *Arabidopsis* hypocotyl. (B,C) cross-sections of the nuclei-localized *H3.1-mCherry* expression under control (B) or circumferential stress (C) using 31-day-old seedlings. 5 biological replicates were included. (D) Quantification of the nuclei number (in the shaded area in B or C) of the *H3.1-mCherry* line under control or circumferential stress (n=5). (E,F) *pPXY::ER-mTurquoise* (a), *pSMXL5::ER-Venus* (b) hypocotyl cross-sections under control (E) or exogenous mechanical stress treatment (F) using 31-day-old seedlings, where the images on the right are the magnifications of the shaded areas within the channel-merged images on the left. 5 biological replicates were included. Scalebars 50 μm .

Figure 5.10: Microtubule orientaton differs in compressed versus non-compressed regions and individual cells grow anisotropically in simulations under confinement. Related to Figure 5.5. (A) Longitudinal sections of hypocotyls under circumferential stress (left) or radial stress (right) using 34-day-old *pWOX4::mVenus-MBD*. Green, mVenus signal; (B) Quantification of CMT anisotropy in cambium stem cells under circumferential stress or radial stress. 3 biological replicates were included. n=28 cells, $P < 0.0001$. Scalebar 20 μm . (C,D) Tissue under standard growth conditions (C) as in Figure 5.3B. and under the same growth conditions except for confined growth via an external, repulsive potential (D) as in Figure 5.5B. (E) Zoom-in of (C) and (D) showing increased circumferential stress at the quadrants next to the applied potential barrier and increased radial stress at the top and bottom quadrants. (F,G) Tissues shown in (C,D) with indication of direction of largest growth (red lines, comparing the difference between $t=0$ and $t=10$) and short axes (green lines w.r.t. $t=10$). (H) Zoom-in on (F,G), showing the largest growth to reorient vertically in response to the external potential. Note that for the purpose of evaluating the largest direction of growth in F,G,H no cell divisions were included in the simulation displayed here.

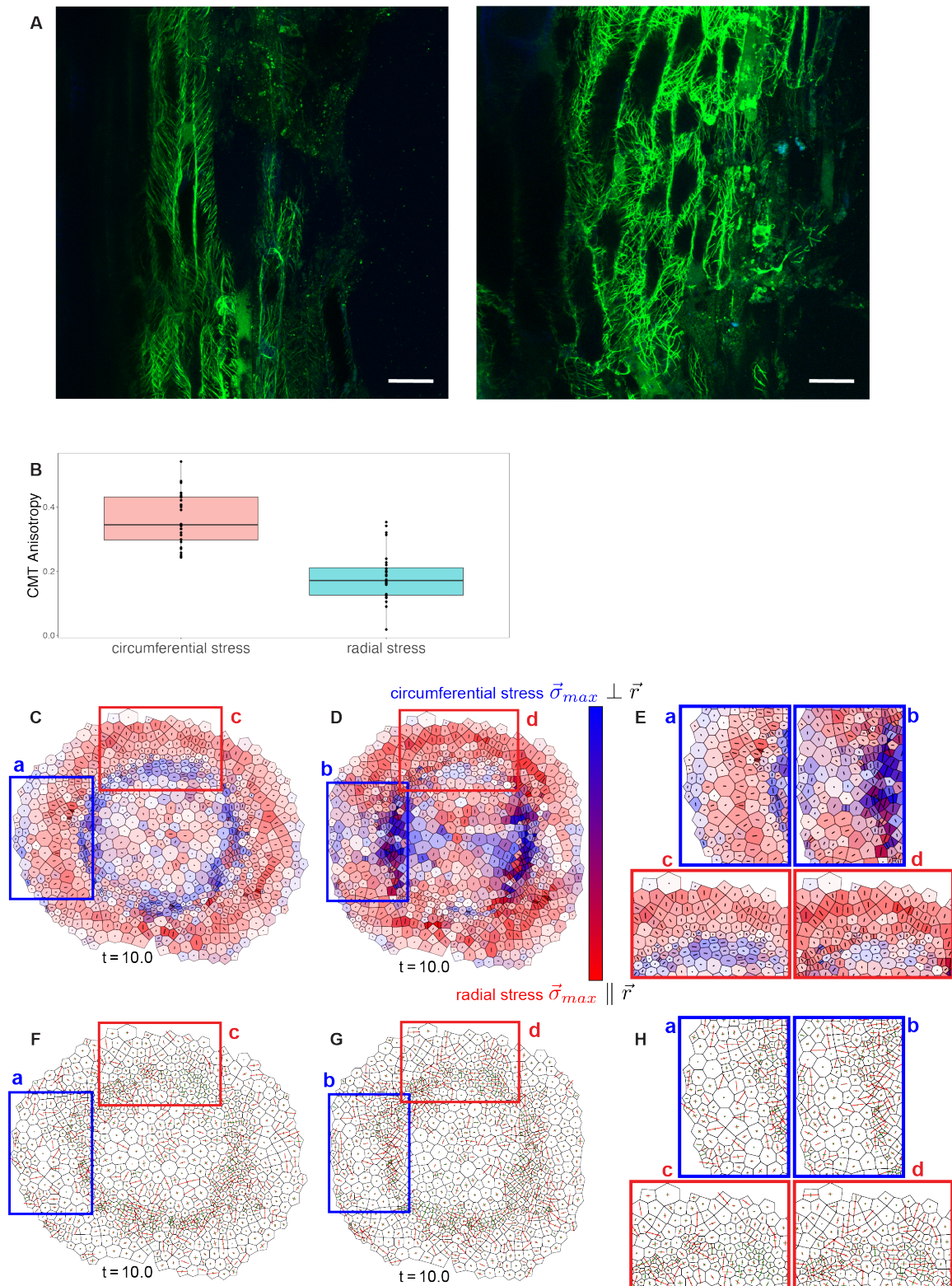
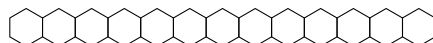


Figure 5.10

6

Conclusion and outlook

*This chapter recapitulates the key insights gained during this work from modeling radial growth and cell division in the hypocotyl of *Arabidopsis thaliana*, integrating plant biology, physics, and mathematics. We set our findings into perspective toward other work and lay out future research directions, including refinements of our model and the integration of biochemical signaling. We provide an outlook regarding possible generalizations toward studies of other plant organs and systems and their potential impact.*



Undoubtedly, many recent findings in plant science have been achieved by interdisciplinary approaches, combining knowledge and methods from biology, physics, and mathematics, amongst others^{18,20,23,67,87,90,91,159,194,211,256–259}. This work contributes from the perspective of computational biological physics to unveil the bio-mechanics of secondary growth in the hypocotyl of *Arabidopsis thaliana*. We have mapped out and validated a mechanical model of radial growth, focusing on stress patterning, cell division, and the response of tissue development to variations of the mechanical properties and growth conditions. In this endeavor, we presented a mathematical description for realizing these processes within a modeling framework, applying the well-established vertex models^{67,68,89,130,191,193,194,257}. We evolved the model from the fundamental aspects of topology and geometry to suit the requirements for simulating radial growth.

By combining the phenomenological shape matrix formalism with infinitesimal strain theory, we established meaningful connections between our model and continuum mechanics. Inspired by plant tissue morphogenesis and experimental analyses, we integrated biophysical processes and observations into the model to ensure and highlight the biological relevance of our study. Such processes include mechanical feedback by cortical microtubules^{28,29}, cell division with maximum stress^{19,69,89,176}, short axis^{79,221,230}, and random division rules, and

the introduction of alterations in the mechanical environment^{23,60,71,177}. Our quasi-static approach of optimizing the tissue energy at each simulation step allowed us to investigate the tissue organization and remodeling dynamics with cellular precision. This level of detail enabled us to capture local variability in cell properties and behavior to replicate and comprehensibly visualize real-world observations in this setting. We benefited greatly in this study from experimental data as a source for necessary assumptions and initial meshes, which we closely related to them. Additionally, we could challenge our results by comparing them with real-world data to put them into perspective and demonstrate our findings' biological significance. Consequently, we established a robust vertex model grounded in insights from both physics and biology. Even though we focused here on the particular context of radial growth in the hypocotyl, our model provides a highly flexible simulation framework that extends to many further contexts, allowing us to capture a variety of phenomena. By the simplicity of adjusting modeling parameters and adding terms to the Hamiltonian, the model is also suitable for simulating other plant organs or animal tissue.

Our findings highlight the significant role of mechanical stresses in the context of radial growth. A key result is the remarkable persistence of the emerging growth and stress patterns from including differential growth by a radial growth factor $\kappa(r/R)$. We found these patterns to be independent of the level of abstraction and variations of the initial tissue topologies and geometries. Despite local perturbations, such as cell ablation or cell division, the global pattern remains stable, and the observed morphological impact agrees with experimental data. This agreement demonstrates the robustness of tissue development, which we successfully reproduced during this study. Even more, we confirmed the hypothesis that the direction of maximal stress correlates with the division plane orientation^{19,69,80,81,176}. Among tested division rules, only the maximum-stress division rule resulted in the emergence of cell files similar to the experimentally observed ones. Other division rules, while not disrupting the stress pattern, would also not give rise to such topologies. Thus, we showed cell files as an emergent result of maximum-stress division, which agrees with our initial hypothesis. While this explains periclinal divisions, anticlinal divisions, crucial for increasing girth during radial expansion, were not captured by maximum-stress alone^{44,83,130,216}. Notably, despite the inclusion of mechanical feedback into the simulations, the short axis of cells in the cambial zone coincides with the anticlinal direction, which matches our experimental observations. While in many cases beyond the present work, mechanical stress is a significant factor behind division wall orientation, geometric division rules have been shown in the past to provide accurate predictions as well^{75–79}. This suggests a dual division mechanism in the hypocotyl of

maximum-stress divisions to produce cell files for radial proliferation and short-axis division to increase tissue girth by producing additional stem cells. Thus, we propose the interplay of both to enhance efficient radial expansion.

Beyond the insights presented here, one key challenge remains: measuring the mechanical properties of tissues and individual cells. While we carefully chose our simulation parameters and mechanical properties to match existing literature, these properties may enormously vary both spatially and temporally in living organisms^{117,260,261}. At the same time, experimental non-invasive methods for measuring the properties of internal tissues like the hypocotyl are still in development^{65,184,262,263}. To date, the highly dense and opaque internal plant tissue refuses to reveal additional insights about its properties without applying destructive methods. Given these constraints, we based our growth dynamics on static cell segmentations. Consequently, we assumed several aspects, like the stiffness of cells, to be implicitly integrated within our radial growth factor $\kappa(r/R)$. Such properties, for instance, are captured by a lower growth rate towards smaller tissue radii, reflecting the higher stiffness of xylem cells compared to phloem cells. Separating different physical aspects of cellular and tissue growth would enhance the accuracy of our model, particularly when comparing *in silico* simulations with *in planta* observations.

Further advances could be made by improving the computational efficiency of the simulations via parallel computing, allowing the consideration of larger tissues and extended proliferation dynamics for a more accurate representation of experimental reality²⁶⁴. While our model accurately represents young hypocotyls, future studies could capture the large-scale proliferation over time in the hypocotyl, considering the dynamics of producing thousands of new cells within weeks⁴⁴. An exciting perspective is to systematically consider the global proliferation dynamics if multiple cell division rules are combined simultaneously into the model, testing our hypothesis of a dual mechanism between stress-guided and geometry-guided cell divisions.

Beyond the refinement of our model, further intriguing studies could extend to the simulation of both transverse and longitudinal cross-sections. To date, a model of cellular resolution for the longitudinal cross-section of the hypocotyl has not been explored, even though it could provide essential insights into the mechanical stress state of cells along this axis.

Internal tissues and the mechanical forces acting on them have been the subject of previous studies^{25,35,40,182,265}. As it stands, the three-dimensional stress state in the hypocotyl remains primarily a hypothesis, except for the outer cell layers, including the epidermis, and a few

internal layers^{25,30,35,60,180,182,183}. However, models that study longitudinal cross-sections already exist to investigate (lateral) roots⁵⁷, and it would be worthwhile to adapt for the hypocotyl. Such a model could then provide insight into whether mechanical stress globally drives the longitudinal divisions, periclinal and anticlinal, observed in the vascular cambium^{42,58}.

If approached with the model presented in this work, some changes would be required, as the shape matrix formalism and its higher-order terms introduce mechanical instabilities from shear stress in highly elongated cells. Mechanisms are required to maintain overall tissue shape and to prevent artifacts unrelated to growth or division. Stability improvements may include external potentials to replicate the stabilization for boundary maintenance, provided by the epidermis, or the transition from a cell-based to a wall-based representation, as demonstrated in previous studies^{57,154,202}. Additionally, inspired by the orientation of cellulose microfibrils, one could consider mechanically anisotropic cells or cell walls, where the dynamics are indirectly controlled via the mechanical feedback due to such anisotropy^{156,266,267}. These approaches may bridge the gap between two-dimensional, cross-sectional models and complete three-dimensional modeling of the hypocotyl. As pseudo- and true three-dimensional models already have been carried out for other plant organs like the shoot apical meristem^{67,68,179,187}, accurate three-dimensional modeling of the hypocotyl only seems to be a matter of time. Thus, the intriguing perspectives of considering all three dimensions will ultimately provide us with a better understanding of the interplay between different tissues and directional cues from the cells' surroundings.

Another future direction would be the integration of biochemical signaling into the computational modeling of tissue and organ development^{256,268,269}. While we did not explore this aspect in our study, there is a great significance of these processes towards the organization and the development of plants as living organisms. Several models have already conducted mathematical and computational modeling on the role of hormones and proteins like, for instance, auxin^{90,236,258,270–275}, expansins^{276,277}, brassinosteroids^{259,265,278,279} and gibberellins²⁵⁹. Such models might offer the opportunity to explore plant development beyond static, predefined implementations of growth and proliferation behavior. Instead, they allow the accurate inclusion of highly dynamic processes and remodeling procedures in plant tissue morphogenesis associated with biochemical signaling pathways. Models of auxin transport^{36,57,59,88,236,237,250,280}, for example, have already shown the potential of considering biochemical patterning, ultimately feeding back onto the mechanical pattern formation. Integrating a true feedback loop between biochemical signals and mechanical properties could

then also allow for realistic modeling of processes like differential growth among others^{26,39,40}. The major challenge is to carefully choose which mechanisms to include and how, in order to obtain a clear and relevant model⁹⁰. This is because individual hormones and proteins highly affect each other²⁸¹, making it challenging to isolate individual contributors due to their interdependence. Despite the challenges, the potential to combine plant bio-mechanics with biochemical signaling remains tremendous and offers exciting perspectives for future investigations.

Plants are highly evolved and developed living beings, even though they may sometimes appear quite inconspicuous. Some have adapted to the seemingly impossible environmental conditions in which they thrive against all odds^{1-4,115,179,248,282,283}. Regardless of all their success in survival and their wondrous versatility, at the very foundation, all their strategies must surrender to the laws of physics across the scales. From the organism level down to the cellular scale, physics embodies the ultimate boundary. Nevertheless, plants have found ways to take advantage of physical principles, and bio-mechanics play an integral role in plant development.

There remains a vast potential in studying plant bio-mechanics and bio-chemistry, whether separately or in combination. Ultimately, the field offers diverse opportunities for future generations of biologists, physicists, mathematicians, computer scientists, and many more to conduct fascinating research and explore fundamental questions. Beyond satisfying individual scientific curiosity, such research may also contribute to addressing future agricultural, ecological, and climatological challenges^{13,119,283-286}.

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Appendices

A

Plant growth modes

A.1 Primary plant growth in roots and stems

Primary plant growth refers to the production of new organs, oftentimes associated directly with elongation growth. The basis for primary growth is thus set during the plant embryogenesis. Here, the embryo undergoes asymmetric cell divisions to establish a so-called apical-basal polarity, which initiates the embryonic organs necessary for further development. These organs and tissues comprise the root, shoot, and cotyledons (Fig. 2.3C), as well as the protoderm, the ground meristem, and procambium as progenitors of the epidermal, cortical, and vascular tissue respectively (cf. Fig. A.1 and Section 2.1.2)^{14,104,118}. At the end of embryogenesis, vegetative growth sets in, and the meristematic tissue in the shoot and the root become active. Fig. 2.3C shows the general structure of an early seedling of the plant *Arabidopsis thaliana*, which is the most widespread plant that is studied in biology due to its simplicity, which makes it a powerful model system⁹³. From top to bottom, i.e., along the apical-basal axis at this point, the plant consists of the shoot apical meristem, the cotyledon(s), hypocotyl, root, and root apical meristem¹⁰⁴. At this developmental stage, primary growth truly sets in. It is mediated by the root apical meristem at the bottom of the plant and the shoot apical meristem at the very tip, respectively, that were formed during the embryogenesis and are therefore called primary meristems^{14,104}. Both permanently grow and potentially produce new organs. The vegetative growth of plants is usually indeterminate, meaning they continue to grow throughout their lifespan, producing new organs, as can be seen with the examples of trees growing for hundreds or even thousands of years in some cases^{12,99}. The shoot apical meristem is crucial for forming new leaves, stems, and flowers, while the RAM drives root growth and development, ensuring the plant can access water and nutrients. They also contribute to forming the periderm, the procambium, and the ground meristem. After the vegetative growth phase, the shoot apical meristem transitions into an inflorescence meristem

that, notably, underlies determinate growth and produces flowers. While the development described so far applies to all plants, there are substantial differences between plant species and groups. Monocots, unlike eudicots, are mostly restricted to primary growth only and often are seasonal or annual plants^{12,14}. As a result, despite the previously mentioned theoretically indeterminate growth, most of these species remain rather small in height due to limits in the structural sturdiness of the organism and a lack of mechanical support.

This limitation usually applies to grass-like plants or grains. However, exceptions, such as bamboo or palms, rely solely on primary growth as well but are still extremely sturdy. Even in palms, secondary vascular development is absent. Instead, they evolved to have sustained primary growth as a strategy of structural reinforcement. This type of growth limits their capability for branching and expanding their crown.

This is because their growth relies on an early establishment of the stem, already at the beginning of the palm's growth²⁸⁷. Thus, already after the early stages, its final diameter does not drastically change any more. In some palms, though, there is an anomalous form of secondary growth where the leaf sheath is wrapped around the stem, resulting in the bases of dying leaves contributing to a rather uniform thickening of the stem. They achieve this by heavily sclerified vascular bundles that provide high stability and elasticity. Still, such strategies differ greatly from those of eudicots, especially perennial species, which are applied to further increase the stem's thickness by producing secondary vascular tissue.^{12,287}

Due to this sole reliance on primary growth, the structure of the plant stems and roots in monocots and eudicots is very different ^a.

In monocots, the cross-sectional tissue structure is very different from that of eudicots. Fig. A.1 displays the tissue structure in both monocots and eudicots during primary growth. Both stem cross-sections consist of dermal, ground, and the just-mentioned vascular tissue that all emerge from the protoderm, the ground meristem, and the procambium, respectively^{12,104,108,113,126,290}. The dermal tissue originates at the shoot apical meristem and protects the internal tissues of both monocots and eudicots. The ground tissue consists of the cortex and pith in eudicots and conjunctive tissues in monocots. It is an organic storage and provides bulk to the stem¹². The procambium vascular bundles consist of xylem and phloem tissue, which we explain in Section 2.1.2. More precisely, these first consist of protoxylem and protophloem, from which metaxylem and metaphloem, separated by the procambium, emerge only after an axial elongation phase. The significant difference lies in the arrangement of the vascular bundles. In the case of eudicots, vascular bundles are arranged circularly in the tissue

^aAs the root morphology is very similar to the hypocotyl morphology, this section focuses mainly on the stems and roots, whereas we give more details on the hypocotyl in Section 2.1.3

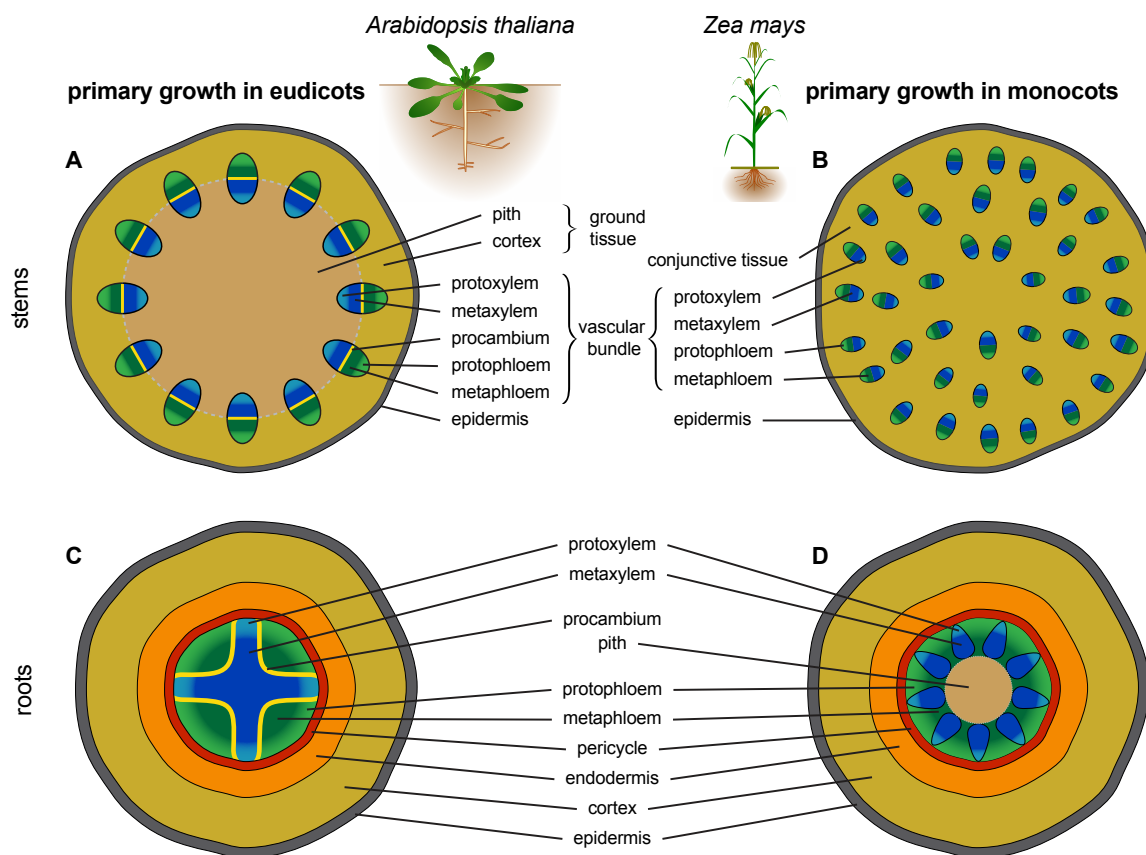


Figure A.1: Schematic lateral cross-sections of primary growth in a eudicot stem (e.g., *Arabidopsis thaliana*) (A,C), and a monocot stem (e.g., *Zea mays*) (B,D) and root: (A) In eudicot stems, vascular bundles are arranged in a circular fashion, consisting of primary xylem (proto- and metaxylem) and primary phloem (proto- and metaphloem), separated by (pro-)cambium. Pith and cortex are distinguishable, and along the radius separating both, interfascicular cambium forms subsequently to primary growth. **(B)** In monocot stems, vascular bundles are scattered throughout the tissue and lack cambial tissue as they do not undergo radial growth. The ground tissue is called conjunctive tissue due to the lack of separability between cortex and pith. **(C)** In eudicot roots, the tissue structure during primary growth highly differs from stems. The primary xylem and phloem are arranged in a polar fashion with two or more poles each, shown in a tetrarch structure. In addition, there are cell layers of pericycle and endodermis, contributing to the radial growth of the root. **(D)** In monocot roots, the primary xylem and phloem arrangement is polyarch with a pith in the center of the cross-section. Drawings of *Arabidopsis thaliana* and adapted drawing of *Zea mays* licensed under Public Domain CC0 1.0 Universal by OpenClipart, SVG IDs 32180²⁸⁸ and 86392²⁸⁹.

(Fig. A.1A), separating pith and cortex. The pith is found in the center of the cross-section and typically consists of thin-walled parenchymal cells that serve as storage in many species, such as potatoes, which store starch and almost only consist of pith^{12,104,108,113,126,290}. The surrounding cortex underlies much variation in thickness and cell types and may serve different purposes depending on the exact plant species. It also consists of parenchymal cells but may include collenchyma with locally thickened cell walls that provide mechanical

support. Furthermore, it may also include sclerenchyma, consisting of dead cells with thick, lignified cell walls that provide very high structural support by their rigidity^{12,99,107}. In monocots, the vascular bundles are scattered throughout the entire stem (Fig. A.1B). Therefore, a distinction between the cortex and the pith is not possible. Instead, the stem consists of conjunctive tissue that features parenchyma or fibers with thick cell walls surrounding the vascular bundles. Embedded within this conjunctive tissue are the vascular bundles. Unlike with eudicots, in monocots, these bundles are scattered throughout the cross-sectional area. Additionally, their internal structure also differs. In both cases, procambial strands produce the xylem and the phloem tissue^{12,84,290}. In monocots, the xylem and phloem reside directly next to each other, surrounded by sclerenchyma. This arrangement is also referred to as closed collateral. As no meristematic tissue is present here, any secondary growth, i.e., radial growth of the tissue, is typically also largely absent. The entire cross-sectional area is established at this very early developmental stage. In eudicots, on the other hand, in between xylem and phloem tissue, vascular cambium originates from the procambium, retaining the meristematic capabilities within these vascular bundles, therefore being referred to as open collaterals^{12,84,94,290}. These are essential for the secondary growth in eudicots, which we will describe in the following section. In roots, the tissue structure is also very different for monocots and eudicots. In eudicots, the roots during primary growth also comprise proto- and metaxylem and proto- and meta-phloem. Unlike in the stem, these have a polar arrangement in pairs of two or more poles of protophloem and protoxylem, which depends on the plant species^{12,93}. In Fig. A.1C, a tetrarch arrangement is displayed. In eudicot roots, there is typically little to no pith¹²³. However, in between the primary vasculature and the cortex and epidermis, an additional layer of pericycle and endodermis surrounds the vascular cylinder. In contrast, in monocots (Fig. A.1D), pith tissue is present, and poles of protoxylem and protophloem are circularly arranged in a polyarch structure. Beyond this, the outer tissue structure during primary growth is essentially identical^{12,93}.

A.2 Secondary plant growth in roots and stems

As described in the previous section, the shoot apical meristem and the root apical meristem drive primary growth and, therefore, are called primary meristems. Primary growth is typically sufficient for most monocot species like grass and grass-like plants. Perennial species, however, rely on increasing their stem's thickness by producing secondary vascular tissue¹². This tissue, which results from radial growth, emerges from lateral meristems and holds great ecological significance. More than half of the world's biomass is wood, and forests provide

habitats for 80% of the world's biodiversity^{93,291}. This secondary growth in eudicots, in general, initiates a transition from a herbaceous to a more woody structure⁹⁹. Such species capitalize on the increase in girth achieved by radial growth to reach much higher structural sturdiness in both the stem and the roots. The increased rigidity allows them to grow to way larger heights and provides them with an advantage regarding the availability of sunlight and amplified capabilities for water and nutrient transport^{32,83,290}.

Before we proceed to describe secondary growth, we want to point out that beyond the primary and secondary meristems mentioned, there are also other types of meristems in plants that do not necessarily directly fall into the categories of primary and secondary growth, even though they are derivatives of it. Notably, primary growth mainly describes the organ formation and elongation growth via the shoot apical meristem and root apical meristem, and secondary growth mainly describes the growth emerging from the vascular cambium and the cork cambium, i.e., the meristems that contribute to the thickening of the stem and roots^{12,14,93}. Beyond this classification, there are other types of secondary meristems in the sense that they emerge after embryogenesis. These meristems include axillary, inflorescence, floral, and intercalary meristems that are, for instance, important for the emergence of branches and leaves^{12,14,93}. Axillary meristems are established by the shoot apical meristem and form at the leaf axils, resulting in small indentations called axillary bud primordium, which allow branching of the stem. These primordia represent a fascinating example of efficient resource allocation in plants. In theory, shoot systems can constantly produce new branches without limits, given appropriate growth conditions. However, most axillary buds do not immediately develop into branches but stay dormant^{99,292} as the shoot apex suppresses their development. This strategy is called apical dominance. For example, this can be observed in *Arabidopsis*, which suppresses the production of branches until after the floral transition, in which it begins producing flowers²⁹². Apical dominance is also a concept that can be seen in everyday life when trimming flowering plants or trees, as once their apex is cut off, they will typically start to produce one or multiple side branches based on the available resources such as access to light, water, and nutrients. Thereby, the whole plant may adapt in response to environmental circumstances by timing the lateral outgrowth or substituting the growth of the shoot apex if it gets damaged^{12,14,93}.

In this work, we focus on one of the secondary meristems, the vascular cambium that is present in both roots and stems^b. During the growth of eudicots, the transition from primary to

^bWhile the cork cambium is also a lateral meristem, we did not carry out any studies towards this meristem, because it is only relevant in mature plants.

secondary growth is directly visible in cross-sections along the length of the shoot. While the length of each section highly depends on the plant species, the upper parts of the shoot axis are still structured the way the shoot apical meristem gave rise to the tissue, as described in the previous section. Further below, towards the base of the stem, secondary growth is visible also in the cross-section, leading to the more woody tissue structure. On a tissue basis, we can discern three growth modes^{93,290}. Each produces secondary vascular tissue in addition to the existing primary vascular xylem and phloem.

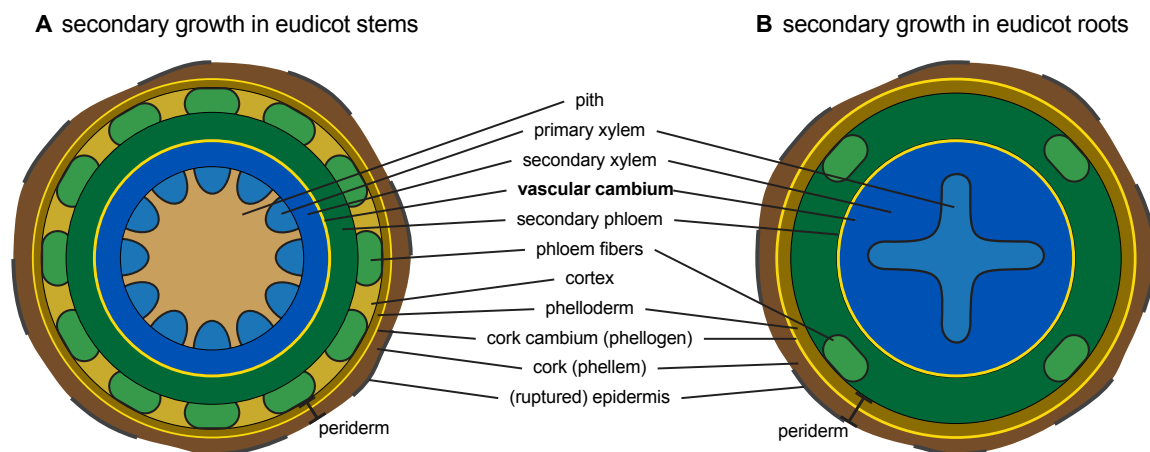


Figure A.2: Secondary growth in eudicot stems (A) and roots (B): (A) In eudicot stems, after the transition from primary to secondary growth, the vascular cambium is established, bi-directionally producing secondary xylem and phloem tissue, resulting in a cylindrical arrangement. As another secondary meristem, the cork cambium (phellogen) also bi-directionally produces tissues. These tissues are the phelloderm and cork tissue, creating the periderm, the “bark” of the stem. The periderm replaces the outer layers, such as the epidermis and possibly other primary cell layers, depending on the origin of the phellogen. (B) In eudicot roots, during secondary growth, as in the stem, the secondary xylem and phloem are bi-directionally produced in the vascular cambium. As with stems, the periderm replaces the rhizodermis (epidermis) as phellogen emerges from the pericycle, bi-directionally producing phelloderm inwards and phellem outwards.

The first one, shown in Fig. A.2, is where the primary vasculature is present in discrete bundles (cf. Fig. A.1, left) that contain fascicular cambium. Later, in the transition towards secondary growth, the interfascicular cambium emerges, connecting the individual vascular bundles to form a continuous ring. The second scenario is that a continuous ring emerges during primary growth, mirrored by secondary growth, i.e., the primary and secondary vasculature take the form of concentric rings. The third possibility is complementary to the previous one, where the primary and secondary vasculature are organized in discrete bundles. While interfascicular cambium is present, it merely forms parenchymal cells from which pith rays emerge that extend from the center of the tissue to the outer layers and transport water and nutrients^{12,93,247}. The vascular cambium bi-directionally produces secondary xylem inwards

and secondary phloem outwards during radial growth^{42,58}. Primary xylem persists due to its strong, lignified cell walls, while primary phloem is typically crushed during outwards expansion. As a result, grown trees mainly consist of dead xylem tissue arranged in annual rings and new phloem tissue is generated to repeatedly replace the previous, crushed one¹². Beyond the vascular cambium, the function and details we will discuss in Section 2.1.2, there is another lateral meristem, the cork cambium, also called phellogen. In herbaceous plants and monocots, typically, the epidermis is the main barrier between the internal tissues and the outer environment and is established during primary growth by the protoderm¹². In the stems and roots of woody eudicots, the periderm fulfills this task. The periderm is a secondary tissue that develops from the pericycle in roots or, varying in different species or organs, from the epidermis, cortex, or secondary phloem by differentiating into meristematic tissue, which is the cork cambium (phellogen, Fig. A.2)^{12,34,108,126,129}. Like the vascular cambium, the phellogen produces secondary tissues, the cork (phellem) outwards and the phelloderm, sometimes called secondary cortex, inwards^{12,108,129,284,293}. Depending on the species, especially in the roots, the phellogen mainly produces phellem. Therefore, tissue growth is primarily unifacial¹². During radial growth and expansion of the plant's cross-section, the epidermis ruptures and is replaced by the periderm. A key difference between the vascular cambium and phellogen is that while vascular cambium persists throughout the plant's life cycle, phellogen is typically only seasonal and newly differentiates every time¹². In the stem, in perennial species, the phellogen is often superficial, i.e., at or close to the surface, and only in the following years, by the production of additional layers is found in layers beneath the surface. In roots, on the other hand, due to the emergence from the pericycle, it is situated further inside from the beginning^{12,126,129}. During the long-time growth and development, plants undergo further morphological changes, production of new, secondary tissue layers, sometimes replacing others either by differentiation or crushing, the details of which depend again on the species¹².

B

Supplementary Results

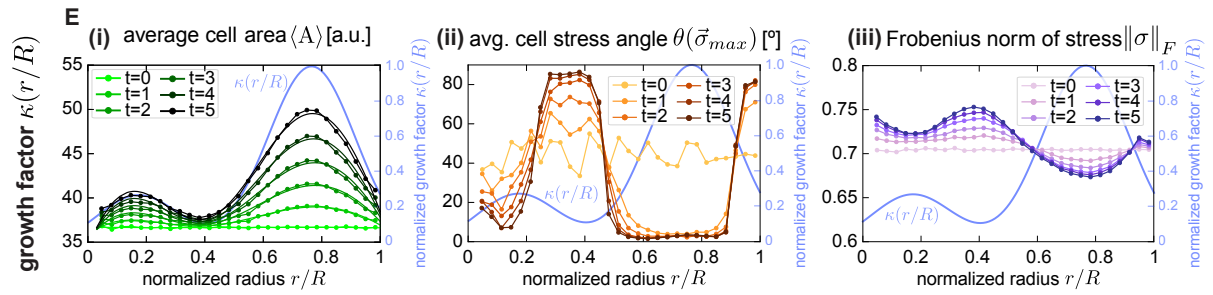


Figure B.1: Addendum to Figure 4.4E. Quantitative analysis of hexagonal tissue growing with growth factor $\kappa(r/R)$. Analysis of areas, stress angles, and stress magnitudes that emerge from applying a radial growth factor $\kappa(r/R)$. In addition to main Fig. 4.4E, each plot includes the radial growth factor (blue line with the axis on the right), showing the overlap of the growth modulation or inverse behavior respectively in comparison with cell areas, stress angles, and stress magnitudes. Related to Fig. 4.4E.

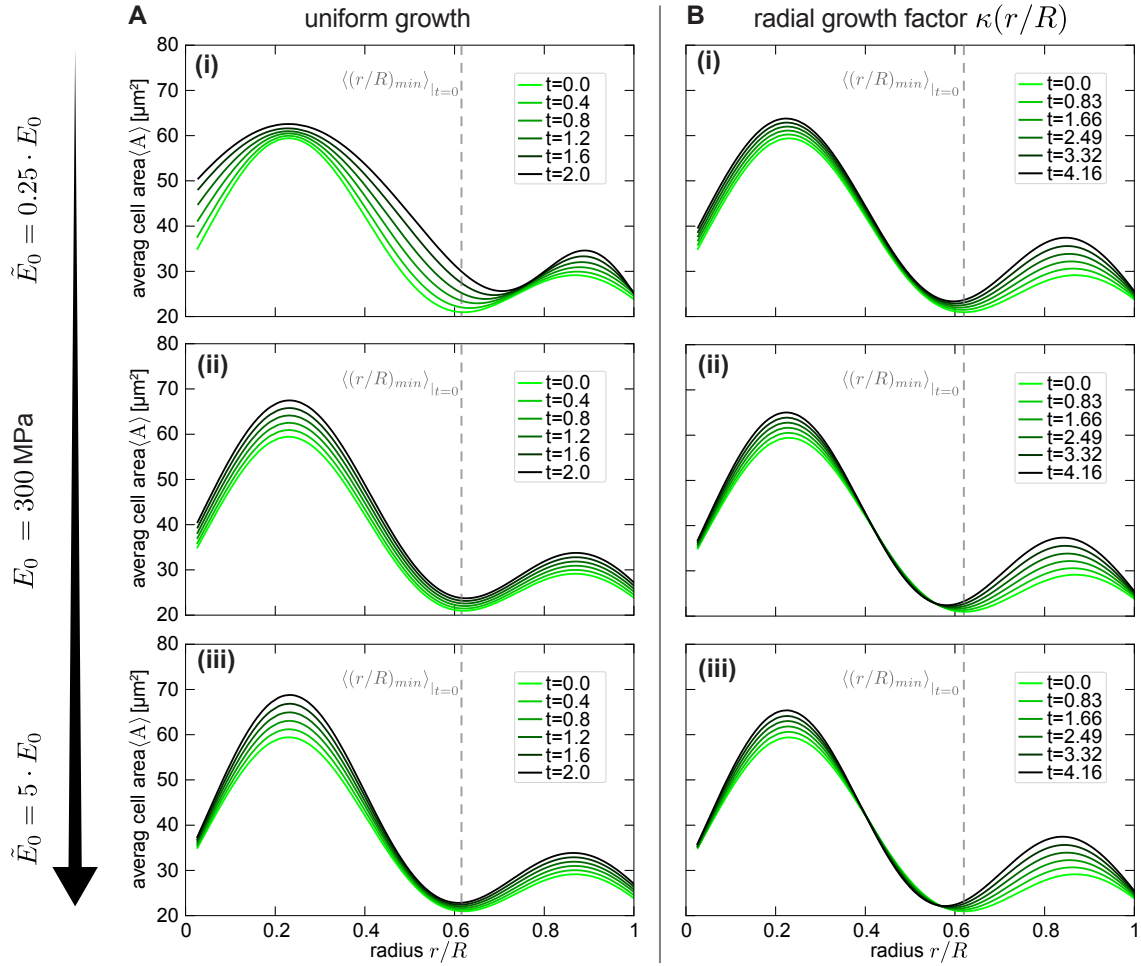


Figure B.2: Area distributions of uniform growth and growth modulated by a radial growth factor $\kappa(r/R)$ as a function of the relative radius r/R under different mechanical properties of the cambium cells.

(A).(i)-(iii) Gaussian fits of the average cell areas $\langle A \rangle$ at different simulation times under uniform growth, demonstrating the effect of lower or higher stiffness of cambium stem cells, ranging from factors of $0.25 \cdot E_0$ to $5.0 \cdot E_0$.

(B).(i)-(iii) Gaussian fits of the average cell areas $\langle A \rangle$ at different simulation times under modulation by a radial growth factor $\kappa(r/R)$ (cf. Fig. 4.6C).

The gray dashed lines in all panels indicate the local minimum at $t = 0$, representing the center of the cambium region, which shifts over time. Related to main Fig. 4.8.

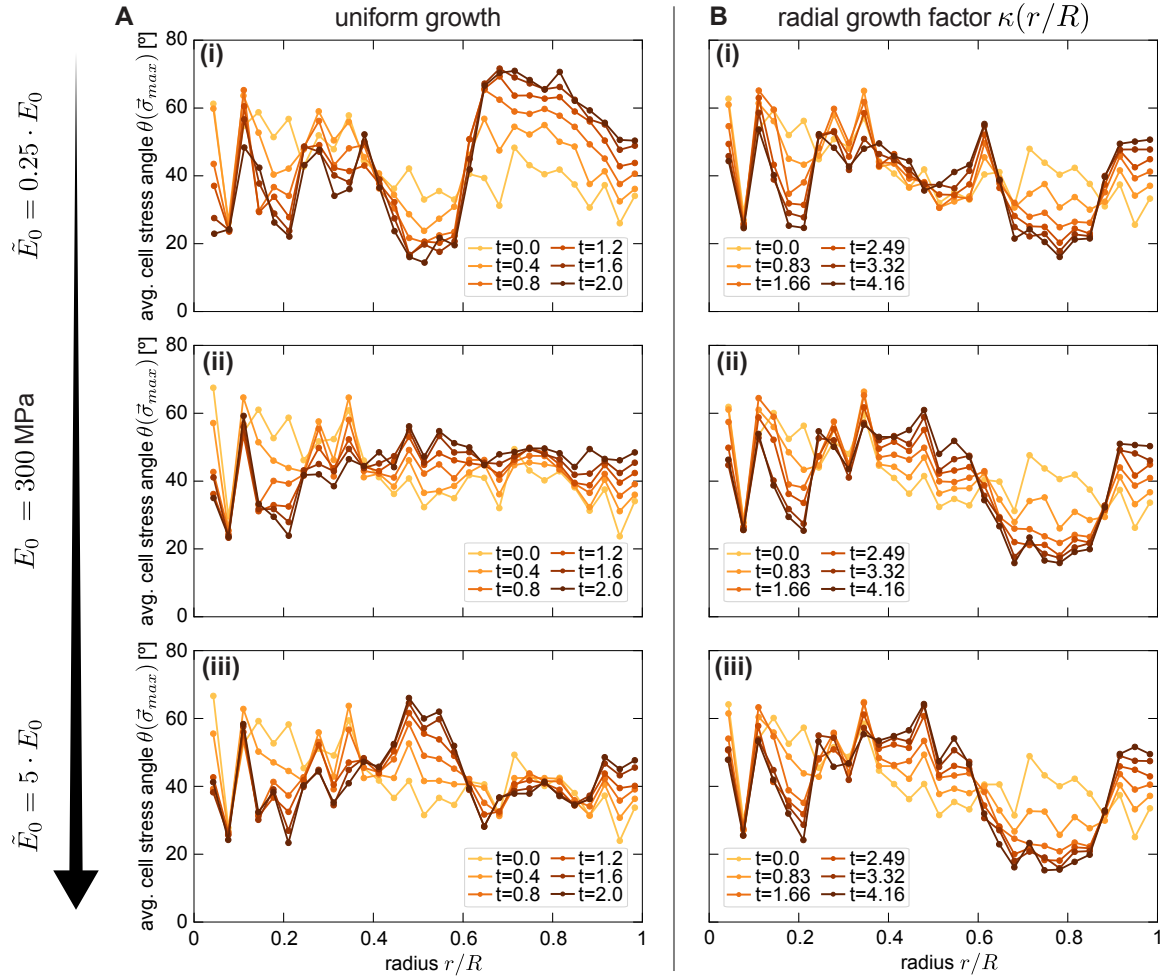


Figure B.3: Angle distributions of uniform growth and growth modulated by a radial growth factor $\kappa(r/R)$ as a function of the relative radius r/R under different mechanical properties of the cambium cells.

(A)(i)-(iii) Binned average stress angles $\theta(\vec{\sigma}_{max})$ at different simulation times under uniform growth, demonstrating the effect of lower or higher stiffness of cambium stem cells on stress orientations. The stiffness ranges from factors of $0.25 \cdot E_0$ to $5.0 \cdot E_0$. A significant change in the stress pattern can be observed for uniform growth with a lower or higher stiffness of cambium.

(B)(i)-(iii) Binned average of stress angles $\theta(\vec{\sigma}_{max})$ at different simulation times under modulation by a radial growth factor $\kappa(r/R)$ (cf. Fig. 4.6C). Circumferential stress in the cambium is slightly suppressed for a low cambium stiffness, while for higher cambium stiffnesses, it is marginally reinforced. Large fluctuations in all panels at low radii result from the low statistics at small radii and the absence of patterning in the very center of the tissue. Related to main Fig. 4.8.

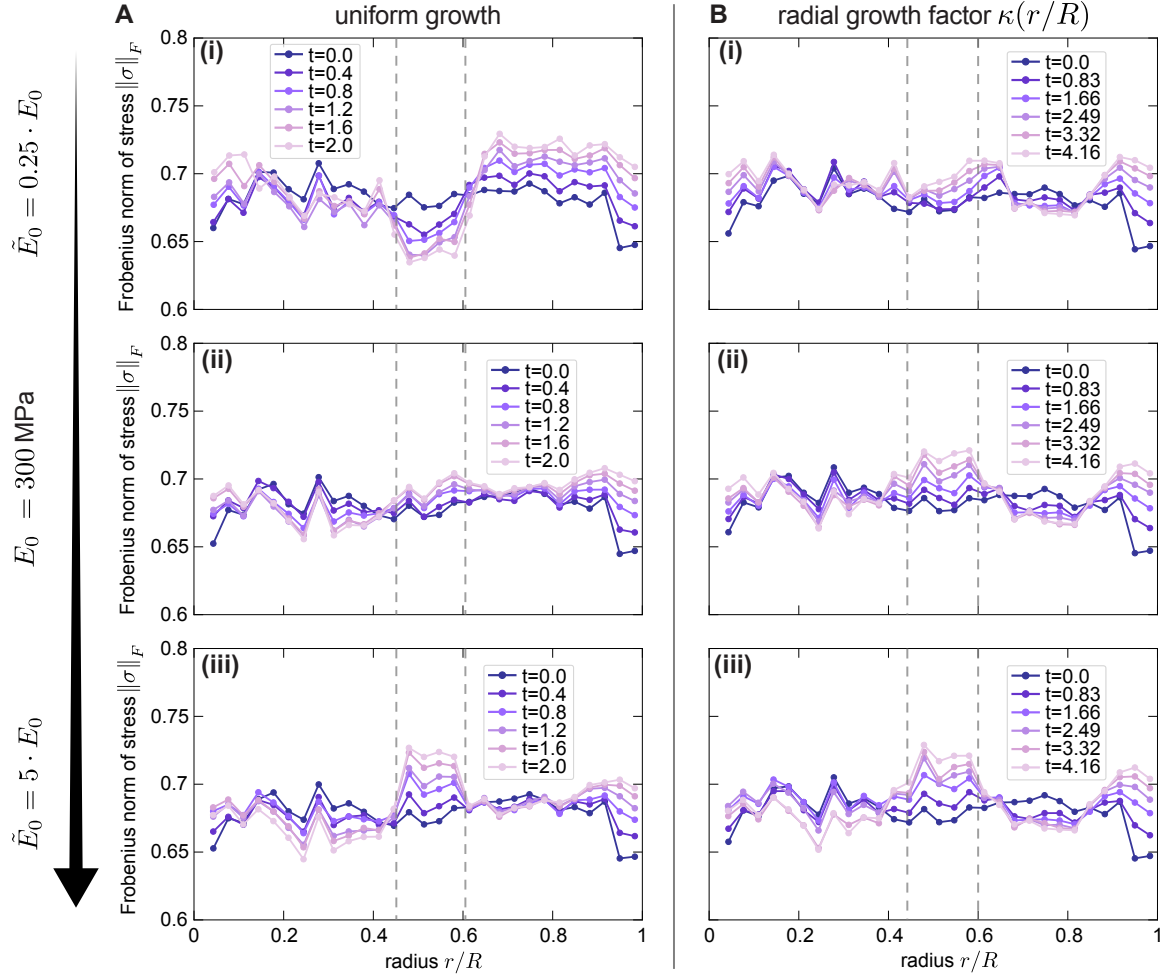


Figure B.4: Binned average of Frobenius norms of stress tensor σ as a function of the relative radius r/R for uniform growth and growth modulated by a radial growth factor $\kappa(r/R)$ under different mechanical properties of the cambium cells.

(A) Magnitudes of mechanical stress for different stiffnesses of factors $0.25 \cdot E_0$, $1.0 \cdot E_0$, $5.0 \cdot E_0$ in cambium cells. For the reference stiffness of $E_0 = 300$ MPa, mechanical stress is rather uniformly distributed, whereas, for lower or higher stiffnesses, mechanical stress in the cambium is increased or reduced. For low stiffnesses, due to the expansion of the cambium, increased circumferential stress is imposed onto the phloem.

(B) Using a radial growth factor $\kappa(r/R)$ as growth modulation, the increased stress in the cambium is largely removed for a lower stiffness of $\tilde{E}_Y = 0.25 \cdot E_0$ and increases for higher stiffnesses. Gray, dashed lines surround the biggest differences in total stress when changing the stiffnesses of cambium cells. Related to main Fig. 4.8.

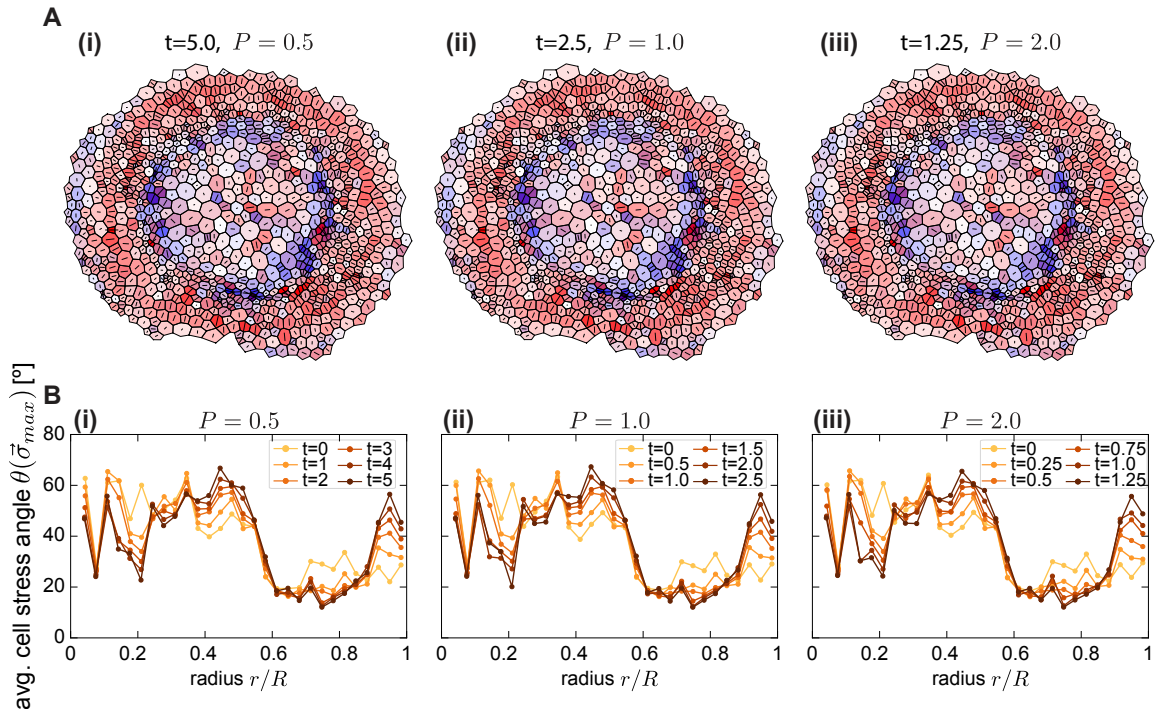


Figure B.5: Tissue growth with changing the turgor pressure in combination with the simulated time yields the same qualitative and quantitative results.

(A).(i)-(iii) Qualitative comparison of stress patterns on the tissue from different combinations of simulation times and turgor pressure.

(B).(i)-(iii) Time evolution of the average cell stress angle w.r.t. the relative radius r/R for the same parameters as in (A). With the given combination of parameters, there is no difference in the quantitative results, except for stochastic fluctuations during tissue growth. Related to main Fig. 4.9.

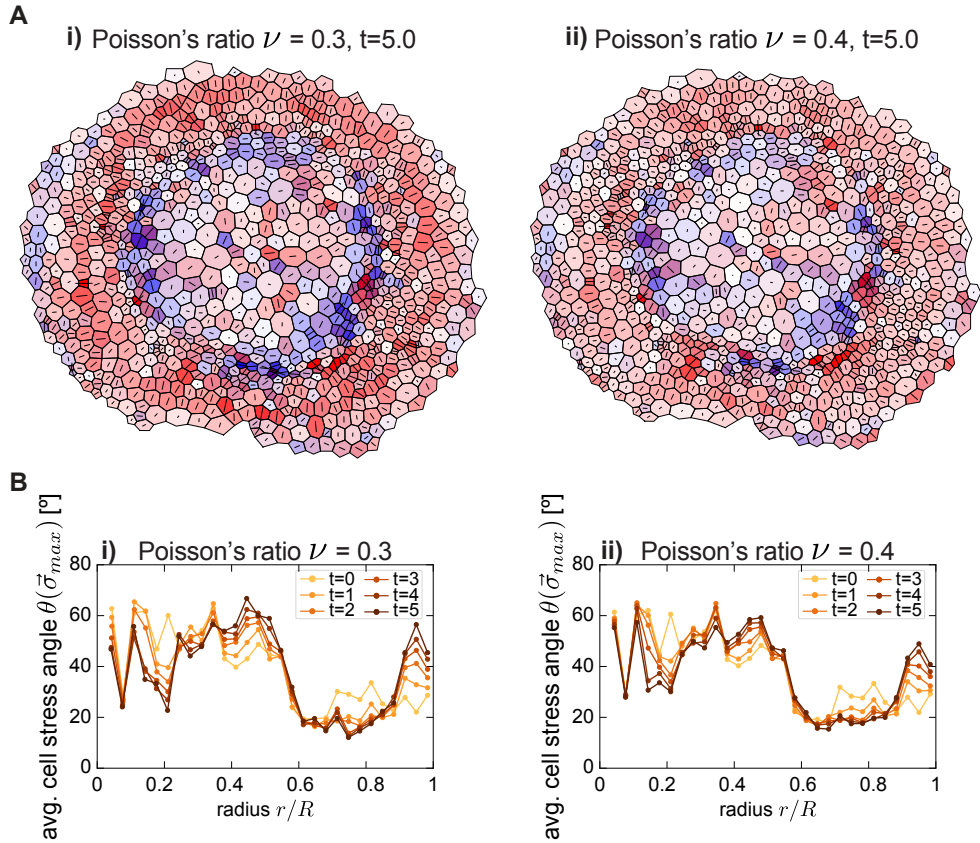


Figure B.6: Changing Poisson's ratio qualitatively does not change the observed stress pattern.

(A).(i)-(ii) Comparison of tissues grown for the same amounts of time at different Poisson ratios. The stress pattern is slightly more intense for a lower ratio of $\nu = 0.3$.

(B).(i)-(ii) Time evolution of the average cell stress angle w.r.t. the relative radius r/R for the same parameters as in (A). The quantitative results are highly similar; however, for a higher Poisson ratio $\nu = 0.4$, the stress pattern is slightly less prominent. Related to main Fig. 4.10



Plant trivia

- Grasses mainly grow their leaves via basal meristems. Therefore, even if the grass is cut, the plant continuously produces new cells⁹⁹, hence requiring the ambitious gardener to regularly mow their lawn.
- Most "aquatic" plants are not actually aquatic but rather land plants that evolved to adapt to permanent submersion under water⁹³.
- Careful grafting of plants can allow for a single plant that grows both potatoes underground and tomatoes above ground¹².
- Paper is produced by de-lignifying wood to get imperforate and perforate tracheary elements, i.e., fibers. For printing, hardwood from, e.g., angiosperms are used as these are finer and shorter; for paper bags and cardboard, softwood from gymnosperms is used¹².
- Maple syrup results from tapping into the starch storage of xylem parenchyma cells in maple trees¹².
- Arnbjerg-Nielsen et al. conducted literal cutting-edge science in 2024 by finding out the required physical parameters of paper to induce paper-cuts to blissfully unaware researchers²⁹⁴.
- The general plant cell types are especially prevalent in several fruits and vegetables. Potato tissue consists almost exclusively of parenchyma cells; in celery stalks, strands of collenchyma cells are visible by the eye in its cross-section, and sclerenchyma cells are found when cutting open pears¹².

Final note

This thesis was typeset with $\text{\LaTeX}$, using the adapted template of Tobias Scheipel, licensed under Creative Commons CC BY 4.0²⁹⁵.

C++ code developed in this work was written using the Eclipse IDE for C/C++ Developers (version cpp-2022-09-arm64)²⁹⁶. Python code was written using Visual Studio Code²⁹⁷. For the mesh creation, the software MATLAB²⁹⁸ was just in addition to Python and C++.